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Review Article

Electropenetrography: A New Diagnostic Technology for Study of Feeding Behavior of Piercing-Sucking Insects†

Elaine A. Backus[1], Po-An Lin[2], Chung-Jan Chang[3,4], and Hsien-Tzung Shih[5,*]

Abstract

Backus, E. A., P. A. Lin, C. J. Chang, and H. T. Shih. 2016. Electropenetrography: A new diagnostic technology for study of feeding behavior of piercing-sucking insects. J. Taiwan Agric. Res. 65(3):219–237.

Studying the feeding, plant damage, and transmission (i.e., acquisition, retention, and inoculation) of plant pathogens caused by hemipteran insect pests has always been a challenge. This is primarily because their specialized, piercing-sucking mouthparts, the stylets, perform probing/penetrating action into opaque plant tissues in which the stylets cannot be directly visualized. This challenge was overcome by a technological revolution over 50 years ago; the invention of electropenetrography, or electrical penetration graph (EPG) monitoring, the most rigorous method to identify feeding behaviors of hemipteran crop pests. Today, EPG is used in three main areas for the development of novel integrated pest management (IPM) tactics for hemipteran pests. Firstly, in cases where the fundamental mechanisms of feeding damage or transmission of a plant pathogen are unknown, EPG is instrumental in identifying such mechanisms. Secondly, once the feeding-related causes of damage or pathogen transmission are understood, EPG can be used to demonstrate the effects of insecticides, antifeedants, or other chemical compounds on specific feeding behaviors responsible for the damage or transmission. Thirdly, EPG can similarly identify the effects of resistant vs. susceptible varieties of crop plants, including transgenic plants genetically engineered to express biopesticides. The purpose of this paper is to review: (1) principles and history of EPG, especially development of the new and improved, third-generation AC-DC monitor, (2) what waveforms and types of information can be gained via EPG, using aphid pests as a model system, and (3) how EPG can be applied to the special needs of Taiwanese agriculture, especially for some species of aphids of economic significance in Taiwan. For the first time in print, our review describes and discusses in details the information of all three types of EPG monitors used for researches on aphids.

Key words: Electrical penetration graph (EPG), Hemiptera, Feeding behavior.

INTRODUCTION

Studying the feeding, plant damage, and (especially) transmission (i.e., acquisition, retention, and inoculation) of plant pathogens caused by hemipteran insect pests has always been a challenge. This is primarily because their specialized, piercing-sucking mouthparts, the stylets, perform probing/penetration into opaque plant tissues in which the stylets can-

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not be directly visualized. Early studies of hemipteran feeding were forced (by the technical limitations of their time) to study snapshots of feeding, after the fact. For example, one could view deposits of hardened saliva *in planta* left behind after feeding activities had ceased (Leopold *et al*. 2003), in essence, frozen in time. Or, one could quantify collections of excretory droplets (Dugravot *et al*. 2008) or document transmission of plant pathogens from long-past stylet probes (Purcell *et al*. 1979; Almeida & Purcell 2006). However, a researcher could not directly study or quantify hemipteran feeding in real time, as it was occurring, until the invention of a revolutionary technology over 50 years ago. That technology is electropenetrography, also known as electrical penetration graph (both names abbreviated as EPG) monitoring of insect feeding (Walker 2000; Almeida & Backus 2004; Backus *et al*. 2005). Most advances in understanding hemipteran feeding behavior have been made possible by use of EPG over the last 50 years.

Today, EPG is used in three main areas for the development of novel integrated pest management (IPM) tactics for hemipteran pests, especially vectors of plant pathogens. Firstly, in cases where the fundamental mechanisms of feeding damage or transmission of a plant pathogen are unknown, EPG is instrumental in elucidating such information. Secondly, once such mechanisms are understood, EPG can be used to demonstrate the effects of insecticides, antifeedants, or other chemical compounds on specific feeding behaviors responsible for damage or transmission. Thirdly, EPG can similarly identify the effects of resistant vs. susceptible varieties of crop plants, including transgenic plants genetically engineered to express biopesticides. Rapid computerized analysis of EPG data can provide quantitative comparisons of, for example, the responses of vectors to resistant and susceptible plants. A researcher can then predict whether a pathogen will be transmitted from/to a putatively resistant host plant, leading to a novel mechanism for host plant resistance.

The purpose of this paper is to review: (1) principles and history of EPG, especially the development of the new and improved, third-generation AC-DC monitor, (2) what waveforms and types of information can be gained via EPG, using aphid pests as a model system, and (3) how EPG can be applied to the special needs of Taiwanese agriculture, especially for seven species of aphids of economic significance. For the first time in print, our review describes and discusses in details the information of all three types of EPG monitors used for research on aphids. Future papers will review the use of EPG in studies of leafhopper, planthopper, psyllid, and whitefly feeding in relation to Taiwanese agriculture. Some of the narrative herein on EPG principles is excerpted and/or adapted from (Backus in press), to which the reader is referred to for more information.

HISTORY AND PRINCIPLES OF ELECTROPENETROGRAPHY

Basic principles. As diagrammed in Fig. 1A, the insect is made a part of an electrical circuit by attaching a thin (~10–60 μm) gold wire to its dorsal surface with conductive glue or paint, then connecting the insect to the input of a head stage amplifier attached to a monitor that also electrifies the plant (Vs to the electrode in the plant soil, Fig. 1). When the insect's stylets probe the plant tissues, fluids in the stylet food and salivary canals ionically conduct the electrical signal through the insect to the monitor, where it is amplified (secondary signal processing circuit, Fig. 1) and outputted to a computerized digital display (not depicted in Fig. 1). Variable biopotentials (i.e., biological voltages or emf [see below] in Fig. 1B) and/or electrical resistances to fluid flow (Ra [see below] in Fig. 1B) generated by the insect-plant interface instantaneously transform

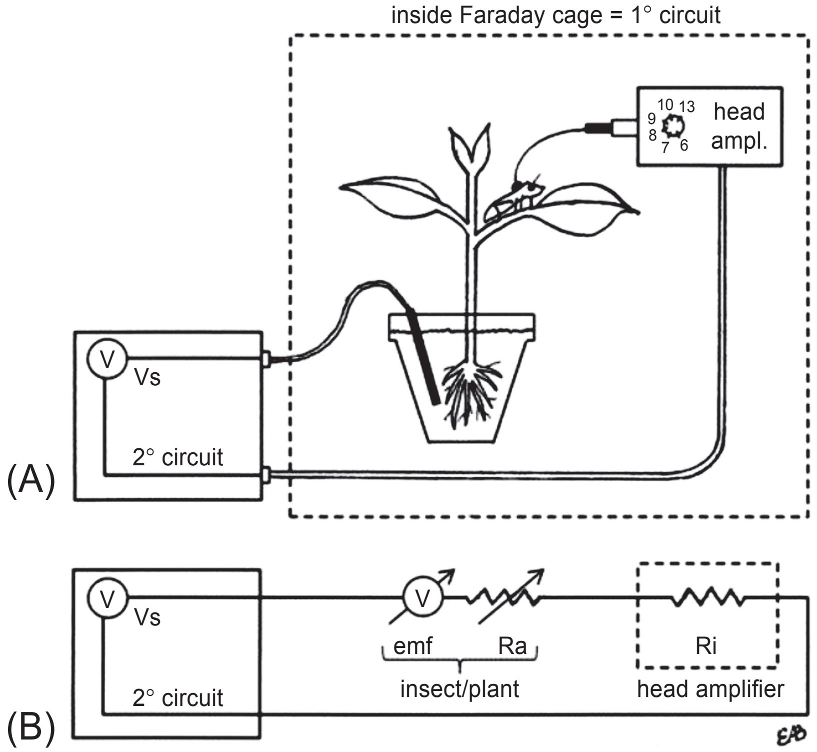

Fig. 1. Diagrammatic representation of the primary (1°) circuit of an EPG monitor. (A) Realistic model of the plant and insect. (B) Electronic block diagram of primary circuit, including variable biopotentials (emf) and variable resistance (Ra). 2° = secondary circuit (i.e., signal processing circuitry); head ampl. = head stage amplifier; emf = electromotive force (biopotential); Ra = insect (e.g., aphid) resistance; Ri = input impedance of the head amplifier; Vs = source voltage. (Derived from an original drawing by G. Walker, in Walker 2000. Reprinted with permission of the American Phytopathological Society)

the constant applied signal into a fluctuating-voltage output signal that is graphed over time as a waveform.

History and monitor designs. First developed in the late 1950s to early 1960s, EPG has advanced over the last 50 years along with the revolution in electronics. The earliest, first-generation EPG monitors, developed by McLean and Kinsey, used technology typical of the time, i.e., glass-tube amplifiers in the late 1950s, later evolving into early solid-state transistors by the 1960s (McLean & Kinsey 1964). They also used AC (alternating current) applied signal, and a low amplifier sensitivity or input impedance (Ri) of 106 Ohms (McLean & Weigt 1968), in a design inspired by radio technology, with which McLean was familiar. These early 'AC monitors' with low Ri outputted signals that were caused primarily by electrical resistance to/conductance of ionic charges carried in fluids (e.g. saliva, plant fluids) passing through the stylets (modeled as a variable resistor, Ra, in Fig. 1B) (Walker 2000). Thus, the earliest AC monitors detected for the first time, in real time, vital information such as beginning and termination of stylet probing, saliva secretion and salivary sheath formation (today termed pathway activities), stylet movements such as extension, retraction, and partial stylet withdrawal, and stylet contact with vascular tissues such as phloem and xylem (Backus *et al.* 2005). Some AC monitor designs actually could detect a small fraction of the biopotentials (voltages) in the circuit (emf,

defined below) (Backus *et al*. 2000); however, practically speaking, AC monitors outputted virtually no emf, due primarily to low Ri level and the then-prevalent use of slow-response strip chart recorders, but also secondarily to bandpass filters found in certain AC monitor designs (Backus *et al*. 2000; Tjallingii 2000).

By the late 1970s, electronics had been revolutionized with improved solid-state transistor technology (by then termed operational amplifiers, or op amps), so that more sophisticated amplifiers and recording devices were available and affordable. The design of the second-generation electropenetrography (termed 'DC monitor' or 'DC system') was introduced by Schaefers (1966), but greatly improved by Tjallingii (1978). Tjallingii used op amps in simple printed circuits, DC (direct current) applied signal, Faraday cages to control noise, FM tape recorders or rapid-response strip chart recorders as output devices, and (most importantly) higher amplifier sensitivity (Ri of either 109 or 1013 Ohms) (Tjallingii 1978, 1985a). Tjallingii's elegant analyses (Tjallingii 1978, 1985a) also established the modern theoretical understanding for EPG science by introducing the concepts of the R (or resistance) component (Ra, described above) and the electromotive force, emf (nearly synonymous with biological voltage or biopotential) component, blended together in the output signal (Walker 2000). The R and emf components are also termed electrical origins of a waveform, and are the foundation of modern EPG science.

There are two known mechanisms underlying the emf component in the plant or plant-insect interface. The first is disruption of the plant cell membrane's charge separation between external and internal cell environments, by stylet tips breaking living plant cell membranes. Such membrane breakages lead to two electrical effects on waveform output: (1) sudden voltage drops as the stylets puncture a membrane, and (2) positive or negative voltage levels that indicate extracellular (apoplastic) vs. intracellular (symplastic) stylet tip positions, respectively. The second biopotential mechanism is streaming potentials, i.e., tiny voltages developed by charge separation that occurs nearly instantaneously in ionic fluids rapidly moving through thin capillary tubes, such as the stylet food and salivary canals (Walker 2000). Streaming potentials cause regular-frequency waveforms that result from rhythmic pumping of muscles (Walker 2000), such as the cibarial dilator muscles controlling uptake of fluid into the functional foregut (Dugravot *et al*. 2008). Increased Ri level in the DC monitor (compared with AC monitor designs) made it possible to detect both R components (previously detected by the AC monitor) and additional emf components in the EPG output waveform. Identifying the electrical origin(s) of a waveform greatly aids in defining its biological meaning.

Another valuable theory of Tjallingii was the sigmoidal emf responsiveness curve (Tjallingii 1978, 1985a), later renamed the R/emf responsiveness curve (Backus & Bennett 2009; Backus in press). The curve is produced when the proportion (0–100%) of emf in an insect's total EPG output signal (Walker 2000) is graphed in relation to Ri level (on a scale of 106 to 1013 Ohms) (Backus & Bennett 2009). The lower the Ri level, the smaller the proportion of the total signal that consists of emf (Fig. 2). Because the ratio of R/emf is reciprocal within the total EPG signal, the smaller the emf proportion, the larger the R proportion (Backus *et al*. 2000; Walker 2000; Backus & Bennett 2009). In addition, the position of the R/emf responsiveness curve with respect to Ri can shift with the size of the insect being recorded [Fig. 2; compare small aphids vs. small leafhoppers vs. large sharpshooter leafhoppers (Cicadellidae: Cicadellinae)]. Generally, the larger an insect's body (i.e., with larger-diameter food and salivary canals, therefore greater ionic conductivity), the more the responsiveness curve shifts towards the left on the chart (Fig. 2). Thus, lower Ri levels allow detection

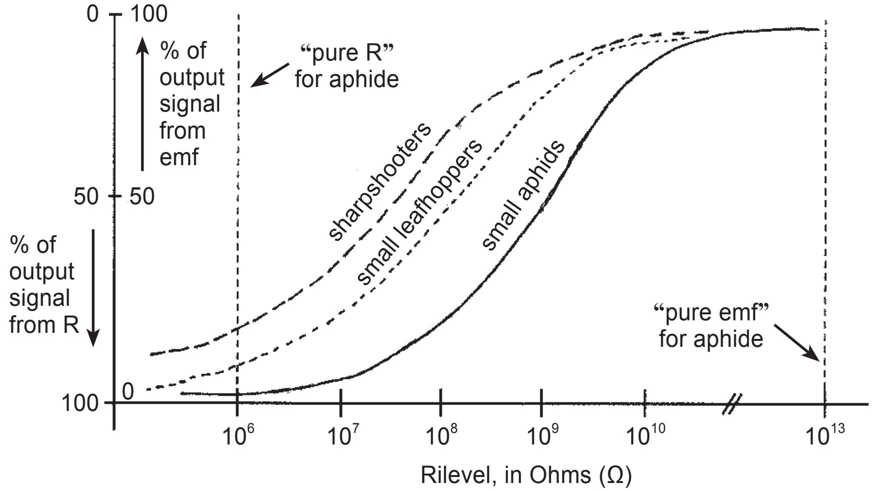

Fig. 2. Theoretical R/emf responsiveness curves for selected example insects. See text for summary, and (Backus & Bennett 2009) for more detailed explanation of the theory. (Reprinted with permission of the American Phytopathological Society)

of more emf in the signal of large hemipterans like sharpshooter leafhoppers, compared with smaller species like aphids. The Ri level that represents the 50:50 R/emf balance point will detect the maximum number of waveforms from both electrical origins, and thus present the best representation of all possible probing behaviors. Tjallingii chose an intermediate Ri level of 109 Ohms for his (DC monitor) design, to balance R and emf components for small aphids. Thus, the R/emf responsiveness curve explains why an AC monitor (Ri 106 Ohms) detected almost no emf component in aphid probing (Backus & Bennett 2009), but slightly more in waveforms of sharpshooters and other large insects (Backus *et al.* 2005).

By the mid-1990s, all EPG researchers were using computerized analog-to-digital waveform display, greatly improving waveform fine-structure detail from both AC and DC recordings (Reese *et al.* 1994). Also by that time, EPG had gradually become specialized so that AC monitors were used primarily for medium-to-large insects such as leafhoppers, planthoppers, and other auchenorrhynchans, while DC monitors were used for smaller insects, especially aphids, whiteflies, thrips, psyllids, and other sternorrynchans (Backus 1994). Such specialization lasted until AC monitors were no longer manufactured (about 2,000); since then, most research has used DC monitors until recently. In retrospect, the R/emf responsiveness curve explains part of the specialization of AC versus DC recordings for differently-sized insects, because signals from small insects like sternorrhynchans would contain both R and emf at Ri 109 Ohms, while recordings of larger insects like auchenorrhynchans would contain both components at lower input impedance (Ri 106 Ohms). However, another reason for past specialization may be due to a difference in tolerance to type of applied signal. Although such differential tolerance is poorly known and an active area of research, anecdotal observations suggest that large leafhoppers like sharpshooters seem to tolerate AC better than DC at low Ri levels, while aphids may tolerate DC better (Backus *unpub. data*). This is important because many R-dominant waveforms can only be detected with rather high applied voltages (hundreds of mV) combined with low Ri (Ri 10^6 or 10^7 Ohms). When high voltages are used, the insect's physiology must tolerate such high current densities. For

some reason (perhaps related to intercellular versus intracellular style of feeding, see below), different insects seem to tolerate DC or AC better at low Ri combined with high voltages.

To increase flexibility for all types and sizes of insects, a third-generation, 'universal' EPG monitor (termed AC-DC) was developed by Backus and Bennett (Backus & Bennett 2009). It provides selectable Ri levels of 106–1,010 plus 1,013 Ohms, choice of AC or DC applied signal, and modern, up-to-date electronics such as instrumentation-quality op amps on standardized, commercially-manufactured printed circuit boards. This instrument removes artifactual voltages and other problems of older electronics (Backus *et al*. 2000; Backus & Bennett 2009), and has controls that are highly sensitive and scalable. Although termed 'AC-DC', the 'universal' instrument is not the same as the old AC monitor technology. The AC-DC monitor was designed to incorporate all functions of the second-generation DC monitor, including retaining emf despite making AC applied signal available (for use with insects that don't tolerate DC at high voltages). For example, the AC signal processing circuit no longer has highpass/bandpass filters, coupling capacitors, log amplifiers, or other components now understood to remove emf from the output signal (Backus *et al*. 2000; Tjallingii 2000; Backus & Bennett 2009). Although rectification is still performed for AC signal processing, a voltage offset knob allows the user to remove the waveform fold-over caused by rectification of negative-going waveforms, restoring perfect waveform fidelity to output of the DC monitor. In addition, the third-generation monitor expands EPG technology to include the first-ever, user-defined choice of Ri levels, very high amplification capabilities, and precise reproducibility of settings with high-resolution dials for gain and applied voltage control, allowing settings to be exactly reproduced among recordings (Backus & Bennett 2009).

As a result of the above design innovations, the AC-DC monitor combines all the advantages of both previous generations of EPG monitor, with virtually none of the disadvantages. Thus, a researcher can precisely tailor the monitor settings to the specific needs of each insect species recorded, allowing characterization of a 'waveform library' of output signals at different Ri levels, more accurately and correctly defining emf- versus R-components (Backus *et al*. 2013; Pearson *et al*. 2014).

Using the AC-DC monitor, it was found that there were no differences in appearance of aphid waveforms based on type of applied signal *per se*, i.e., AC versus DC. However, large differences occurred based on Ri level, as predicted by the R/emf responsiveness curve (Backus & Bennett 2009). These results support the success of the design goals for this monitor. In a similar manner, differences in amplitude among waveforms can be used to demonstrate the effects of the R/emf responsiveness curve, to determine electrical origins of waveforms by changing the Ri level.

Principles underlying biological meanings of EPG waveforms. The biological meanings of EPG waveforms are defined by correlation with: (1) stylet tip locations in the plant (e.g. vascular versus non-vascular tissue termini), via histology of salivary sheaths, cut stylets in probed plant tissues, and/or fluid exudation of cut stylets, (2) intricate stylet activities (e.g., stylet movements, salivation, etc.) performed by the insect, via observation in transparent artificial diets, and other methods, and (3) electrical characteristics of the waveforms, some of which have been explained above (Walker 2000).

Additional electrical characteristics of waveforms include those that reveal stylet depth. This can occur in two ways, depending upon Ri level. Waveforms recorded at lower Ri level (106 or 107 Ohms, depending on species) are usually positive-going, and the position of the waveform above 0 V baseline

(termed voltage level) is correlated with stylet depth (see figures in Backus *in press*). In other words, the voltage level rises or falls as the stylets are partially withdrawn or pushed more deeply into the plant tissue, respectively (Jiang & Walker 2001; Backus *et al*. 2005). Thus, stylet depth in the plant is a strong R component. At higher Ri settings (10^9–10^{13} Ohms), waveforms usually become both positive- and negative-going (Figs. 3A and 3B), and the biological meaning of voltage level shifts so that it reflects the electrical charge immediately surrounding the stylets tips; thus plant electrical charges internal or external to cells create a biopotential, i.e., an emf component. Negative voltage level (termed 'intracellular' level in the DC EPG literature) (Walker 2000) denotes symplastic space (i.e., inside living cells whose charge separation between external and internal environment is maintained by an intact plasma lemma that is either not disrupted by stylet penetration or sealed by sheath saliva). Positive voltage level (termed 'extracellular') denotes apoplastic space in the plant (i.e., intercellular spaces, interior of cell walls between living cell membranes, mature xylem, or living cell interiors whose electrical charge separation has been destroyed by stylet action) (Backus *et al*. 2009; Miranda *et al*. 2009; Pearson *et al*. 2014). Interestingly, at the intermediate Ri level of 108 Ohms with the AC-DC monitor, waveform voltage level can be either positive-going only or both negative- and positive-going, depending upon the insect (i.e., probably quality of wiring job) or probe.

Another general characteristic is presence of a landmark waveform termed the X wave (Backus *et al*. 2009). Originally identified by McLean & Kinsey (1967), an X wave is a complex, highly visible, stereotypically repeating waveform that is species-specific and (to date) performed only by salivary sheath feeders (McLean & Kinsey 1967; Backus & Bennett 2009). An X wave marks contact with and penetration of the stylets into a preferred cell type for eventual, sustained ingestion. Accordingly, a true X wave marks the transition between pathway and sustained ingestion phases; both are performed in an acceptable 'target' cell of the preferred ingestion cell type. For all sheath-feeding hemipterans, X waves provide a visual portrayal of the behavioral acceptance process for an ingestion site. Thus, they represent a series of behaviors that: (1) sensorially test and judge the acceptability of a phloem sieve element or xylem vessel element (depending on the ingestion preference of the species), (2) secure a firm attachment to that cell, and (3) begin overcoming any challenges or defenses presented in the cell by the plant (Backus *et al*. 2009). Identification of the sharpshooter X wave marked the first time that such specialized contact and acceptance behaviors have been demonstrated for xylem vessels by preferential xylem-ingesters; all other X waves have been defined for preferential phloem-ingesters (e.g. aphids, deltocephaline leafhoppers).

APPLICATIONS OF EPG TO APHID BEHAVIOR AND IPM

Since the earliest years of electropenetrography, aphids have been widely used as models for studying hemipteran feeding behaviors. The relatively large size and low mobility of aphids makes them perfect for EPG studies. Today, virtually everything known about aphid feeding behavior can be attributed to EPG. Feeding activities of aphids are composed of several sequential behaviors. After an aphid make contacts with a plant, it antennates, using chemosensory hairs to sense gustatory cues (Powell & Hardie 2002). During the first few probes, an aphid will intermittently puncture living plant cells (Tjallingii 1985b), and plant fluids then are taken up to the precibarium (or antecibarium) to make contact with chemosensilla therein; the aphid then will decide to leave the plant or continue probing into the vascular tissues (Powell *et al*. 2006).

The following review summarizes and

synthesizes information about aphid feeding from all three generations of EPG technology. Today, aphid waveforms are classified into three main phases, including: (1) pathway phase, (2) phloem phase, and (3) xylem phase, plus a transition phase (X wave) that alternates between pathway and phloem. Pathway phase comprises waveforms A, B, C, F, and potential drop (pd) (Figs. 3A–3D) (Tjallingii 1978); phloem phase consist waveform E1 and E2 (Figs. 3E–3H); xylem phase consists only of waveform G (Fig. 3J) (Tjallingii 1987). Although the waveforms can be readily categorized by human eyes, it is difficult to define each of the waveform explicitly. In addition to subjective categorization, crude definitions based on relative amplitude, frequency, and voltage levels are used to characterize each waveform (Tjallingii 1978). Almost all aphid species studied show very similar waveforms, representing distinct, corresponding behaviors. The consistency among aphid species is a powerful advantage for selection of aphids as the model for EPG studies, as well as for application to integrated pest management tactics against these important pests.

Aphid waveforms and their biological meanings:

Pathway phase: A, B, and C. In order to locate and establish sustained phloem ingestion, aphids use the salivary sheath strategy of feeding (thus, secreting both sheath and watery saliva), performing intercellular stylet penetration during most of the stylet probe/penetration (individual stylet insertion), simultaneously secreting sheath and watery saliva (Moreno *et al*. 2011), with intracellular penetration more rarely (Fig. 3A) (Pollard 1973). Onset of a stylet probe usually takes place within 1 min after the aphid makes proboscis contact with the plant (Powell *et al*. 2006). Penetration of stylets causes an abrupt, positive-going voltage change. Following initial penetration, waveforms A, B, and C (Figs. 3A and 3B) are usually observed (Tjallingii 1978). These waveforms are always performed in consecutive sequence, and together are referred to as 'pathway activities' of aphid stylet penetration (Tjallingii & Hogen Esch 1993). A, B, and C were highly compressed and combined as a single waveform (called Salivation) in the early years of EPG, due to the slow speed of then-prevalent strip chart recorders; thus it is unknown whether the three waveform types were distinguishable using the low Ri level of the first-generation AC monitor (McLean & Kinsey 1967). Although the detailed activities underlying A, B, and C are still to be unraveled, their importance prior to sustained phloem sap ingestion is certain. Details about the route and positions of the stylets within plant tissues can be found in Tjallingii & Hogen Esch (1993).

Pathway phase: pd. During pathway, especially waveform C (which represents the majority of pathway), there are often numerous intracellular punctures of cells along the stylet route, represented by potential drops (pd) (Figs. 3B and 3D), which last for 5–15 s. The first pd can take place as soon as 10 s after initiating stylet probing, but it usually occurs within 1 min after penetration (Fig. 3B). Parallel lines of evidence over several papers support that these sudden voltage drops are caused by the puncture of parenchyma or epidermal cell membranes. For example, by comparing waveforms of aphids on artificial diets, protoplast suspensions, and plants, Tjallingii (1985b) was able to correlate waveform pd with the presence of living cells. In addition, the voltage level of pd is usually lower than that of waveform C by 100–180 mV; this voltage level resembles the membrane potential of plant cells (-100 to -200 mV), as well as that of waveform E (discussed below), when stylets are located inside a phloem sieve element (Tjallingii 1982). It was thus concluded that pd was caused by the change of potential from intercellular regions into intracellular ones (Tjallingii 1985b). Further support for the hypothesis occurred when numerous mesophyll punctures were observed along the salivary

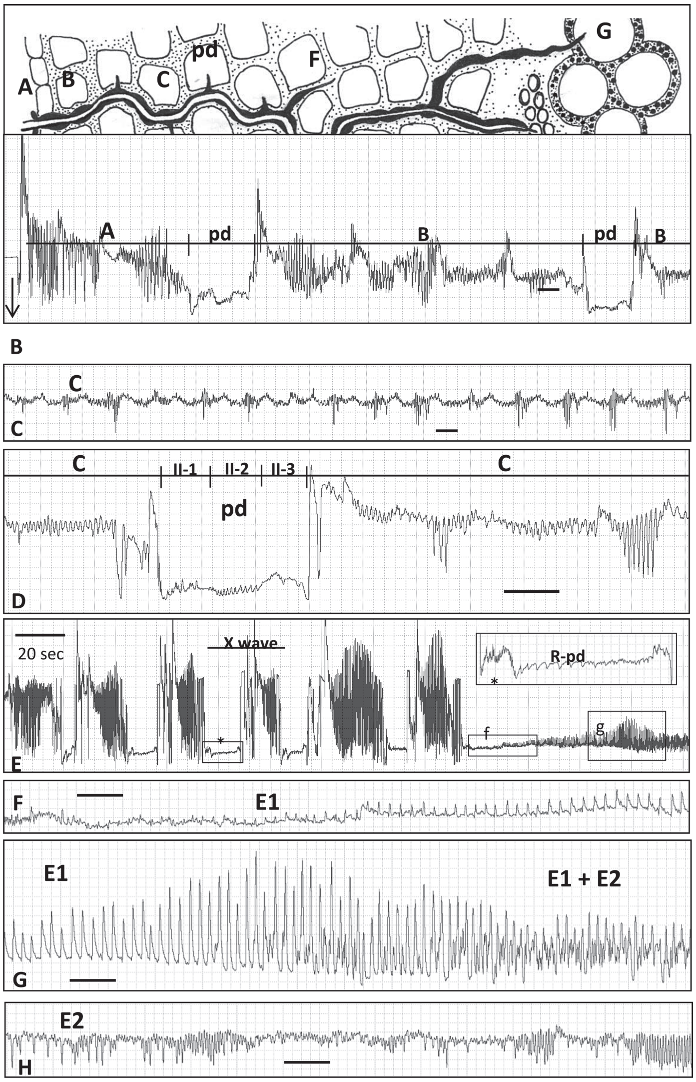

Fig. 3. Summaries of typical EPG waveforms (using an AC-DC monitor at Ri 109 Ohms) from stylet probing of the pea aphid, *Acyrthosiphon pisum*. (A) Drawing of a hypothetical salivary sheath/stylet pathway to xylem and phloem cells with labeled portions of the sheath to represent typical locations of the waveforms (letters) recorded during each stage of probing. The sheath is hollow because the stylets were removed during E2, to identify the final sheath branch into a phloem sieve element cell. Filled, abandoned, branches represent stylet derailment during probing (waveform F; not pictured below) and xylem ingestion (waveform G, not pictured below). All scale bars represent 2 s of recording, unless otherwise stated. (B) Start of a stylet probe (voltage increase after flat baseline/non-probing; baseline denoted by an arrow). Note that waveform A is quite brief, and that the first intracellular puncture (pd) occurred within the first 30 s. The two pd's are also noticeably below the baseline level (therefore, negative-going). (C) Waveform C, much later in pathway; note that its amplitude (Y axis) is shorter than that of either A or B. Amplification of parts b and c is 4×. (D) Short section of waveform C with embedded pd (with pd sub-phases), at twice the amplification (8×) of part c. (E) Compressed view of 5.5 X waves at the end of pathway in the same probe, prior to E1 then E2. Boxed area of waveform with * is enlarged in the inset box, showing details of sub-phase II of the R-pd. Lettered boxes on waveforms are expanded in the next sections. (F) Start of E1, with positive-going peaks, showing the evolution of its appearance over time. Amplification of sections f–h is 8× (double that of section e). (G) Tallest peaks of E1 in transition to E2, during which the E1 and E2 peaks overlap and merge. (H) E2 alone, much later in the probe. An appearance similar to classical E2 (using the DC monitor), with negative-going peaks, occurs at the beginning of this excerpt near the letter h; other variations in appearance are typical of pea aphid recordings with the AC-DC monitor, probably due to greater sensitivity and rapid response time of modern amplifier electronics.

sheath (i.e., route of stylets) using electronic microscopy (Tjallingii & Hogen Esch 1993).

Potential drops are subdivided into three sub-phases (or sub-types), namely I, II, and III (Fig. 3D). Sub-phase I is the abrupt, descending drop of the waveform; sub-phase II is the short period of stable waveform at a lower potential level; sub-phase III is the sudden rise/recovery of potential back to the original (extracellular) level. The activities reflected by pd sub-phases I and III are the penetration of stylets through the plasmalemma and the withdrawal of stylets, respectively (Tjallingii 1985b). Sub-phase II is a more complicated waveform consisting of three distinctive parts, II-1, II-2, or II-3 (Fig. 3D) (Powell *et al*. 1995). Because it is very difficult to correlate stylet activity in a cell within 5–15 s by conventional methods (e.g. stylectomy), different approaches using aphid-transmitted non-persistent viruses as phloem markers have been used to elegantly correlate the behavior of aphids during sections of pd sub-phase II (Martin *et al*. 1997). Emulating the idea from earlier, AC monitor research of Scheller & Shukle (1986) (discussed below), aphid-virus combinations were used from two pathosystems, *Aphis gossypii* Glover transmitting *Cucumber mosaic virus* (CMV) and *Myzus persicae* Sulzer transmitting *Potato virus Y* (PVY). In the first study, non-viruliferous aphids were recorded during an acquisition access period on virus-infected plants, which was terminated at the end of sub-phase II-1, II-2, or II-3 in the first-recorded pd, and the aphid was later used to test inoculation efficiency. In a second experiment (similar to the acquisition experiment), viruliferous aphids were recorded during an inoculation access period, which was terminated at the end of sub-phase II-1, II-2, or II-3 in the first pd. The result indicated that pd subphase II-3 was correlated strongly with acquisition thus indicating intracellular ingestion, and pd subphase II-1 corresponded to viral inoculation thus indicating intracellular salivation. These findings suggest that the behaviors during sub-phase II-1, II-2, or II-3 are likely salivation, unknown behavior, and ingestion of plant cell contents, respectively (Martin *et al*. 1997). Harris later hypothesized that sub-phase II-2 represents egestion of fluid from the precibarium in the functional foregut (Harris & Harris 2001). This hypothesis has not been experimentally tested in aphids, however, the existence of egestion in hemipterans has recently been proven (Backus *et al*. 2015).

The pd waveform is best viewed using high Ri levels, such as with the DC (10^9 Ohms) or AC-DC monitors (10^8 Ohms or higher), because all three sub-phases are visible. However, sub-phase II-2 was also detectable using the AC monitor (Reese *et al*. 2000) at lower Ri level (10^6 Ohms). Sub-phases I and III (the fall and rise) were not visible, but a version of sub-phase II (a mostly-flat line, without divisions into the three sections) was visible. When pd's are recorded at multiple Ri levels using the third-generation AC-DC monitor, the depth of the pd and amount of detail of sub-phase II sections increase as Ri is stepped up from 10^6 to 10^{13} Ohms (Backus & Bennett 2009). This confirms that the sub-phase II details are emf components.

Pathway phase: F. Waveform F is occasionally observed during pathway activities, and it was never recorded in feeding of artificial diet. The duration of waveform F varies from minutes to hours and does not lead to phloem penetration. All the aphid species tested in the study of Tjallingii (1987) perform this waveform, but showed different frequencies among host plants. According to histological studies, the aphid's stylets were always located within the intercellular space of epidermal cells with thick cell walls and obscure middle lamellae. However, results from radioisotope intake experiment shows a ten times higher rate of plant sap uptake during F than during waveform A, B, and C. Based on limited information, waveform F is presumed to be the interaction between stylet and particular cell walls while the stylets are struggling to

free themselves from a tight space (Tjallingii 1987). The waveform is now termed 'derailed stylet mechanics'. Waveform F was termed 'non-phloem ingestion' in studies with the AC monitor, and was not distinguishable from waveform G (see below) using the lower Ri level of that early monitor.

X wave, an inter-phase transition. The first identification of an X wave as a landmark waveform was made with the AC monitor at low Ri level. Although the behavior represented by the X wave was unknown, it was found to precede ingestion from phloem sieve elements by *Acyrthosiphon pisum* Harris, 100% of the time (McLean & Kinsey 1964, 1967). A study of feeding by *Sitobion avenae* F. (Scheller & Shukle 1986) elegantly correlated X waves with inoculation by using the aphid-transmitted plant virus, *Barley yellow dwarf virus* (BYDV) as a marker. BYDV is persistent-circulatively transmitted by *S. avenae* (among many aphids), and is phloem-limited. Virions are acquired by ingestion of phloem sap, and inoculated (after a latent period for virus circulation) via salivation into phloem sieve elements (Sylvester 1980). EPG waveforms were recorded for 90 min while viruliferous aphids were feeding on healthy test plants. Results showed that X waves were prerequisite for inoculation, thus they represented both contact with and salivation into phloem sieve elements. A single X wave resulted in a 65% chance of inoculation and subsequent infection, regardless of the duration of the X wave. The probability of inoculation/infection increased with increasing numbers of X waves (Scheller & Shukle 1986).

A deeper understanding of the relationship between X waves and pd's began to develop when Reese *et al*. (2000) identified the first part of the X wave as a pd that was significantly longer than earlier pd's in the same probe. The second part of the X wave was a return to C with a longer, more elaborate comb-like portion than in pre-X pathway C. Thus, the long-duration components of the X wave made the waveform easily identifiable, even in the highly compressed recordings of the AC monitor. Identification of an unusual form of pd, the R-pd, finally explained the mechanism of the aphid X wave. R-pd's have been observed in *A. pisum, Brevicoryne brassicae* (L.), *Drepanosiphum platanoidis* (Schrank), and *Periphyllus acericola* (Walker) (Tjallingii & Gabryś 1999; Backus & Bennett 2009). This unusual pd is an extended form of normal pd, with longer subphase II and smaller amplitude (Fig. 3E, inset box with *). R-pd is always part of an X wave (Fig. 3E), thus always precedes waveform E1, and occurs more often on mature and old leaves (less preferred feeding sites) than young leaves and stems by *B. brassicae*. Together with the results from electron micrographs, Tjallingii & Gabryś (1999) concluded that the R-pd waveforms (and thus X waves) were caused by repeated punctures into and out of the same sieve element. Accordingly, the aphid X wave represents a transition alternating between pathway and phloem phases. Although the function of the X wave behavior is not yet proven, it is likely to be part of the cell-acceptance process of aphids, as with X waves of other sheath-feeders (Backus *et al*. 2009).

Phloem phase: E1 and E2. Early studies using the DC monitor showed that phloem penetration was associated with waveform D + E (pd) (Kimmins & Tjallingii 1985), which was later renamed and divided into waveforms E1 and E2 (Tjallingii 1990). Usually, E1 begins right after the last X wave; after a certain period of E1, E2 will ensue on a suitable plant. E1 and E2 are composed of very regular, repetitive peaks (Figs. 3E–3H). The amplitudes of waveforms E1 and E2 are low compared to waveforms A, B, and C. The main differences between the two waveforms are their patterns and electrical origins. Waveform E1 is an emf-dominated waveform, whereas E2 is an R-dominated waveform, which means waveform E2 changes according to the adjustment of substrate voltage, and waveform E1 is less affected (Tjallingii 1990). Waveform E2

always occurs after E1, and never the reverse. Although waveforms E1 and E2 seem to be highly associated, performance of waveform E1 does not necessarily lead to E2 (Prado & Tjallingii 1994). Early studies with the AC monitor could not distinguish between E1 and E2, due to low Ri level; the combined waveform was referred to as 'phloem ingestion' due to histological correlation with phloem; however this waveform is now better described as phloem phase.

The biological meanings of waveform E1 and E2 are well understood. They were first associated with sap ingestion and salivation, respectively, based on experiment result using radioactive labeled feeding solutions and electromyograms (EMG) of salivary pump muscle (Tjallingii 1978).In the early DC literature, duration of waveform D (today equivalent to E1 and E2, together) was positively correlated with the amount of radioactive orthophosphate inside aphids, and the frequency of the fluctuation pattern coincided with pattern of salivary pump movement in the EMG. The positions of the stylets were demonstrated by stylet amputation and electron microscopy observation. When aphid stylets were amputated 7 min 30 s after the start of waveform E1 and E2, phloem sap exuded from the cut end of the stylets due to the phloem turgor pressure. Electron micrographs clearly showed that the stylets, during waveform E1/E2, were inside the lumen of sieve element (Kimmins & Tjallingii 1985) and no sheath saliva was found inside sieve element (Tjallingii & Hogen Esch 1993).

In the study of Prado & Tjallingii (1994), they reported that the behaviors during waveform E1 and E2 were correlated with salivation (E1) and ingestion simultaneous with watery salivation (E2) in phloem sieve elements, after repeating and expanding upon the original study of Scheller & Shukle (1986). Again, they used the aphid-transmitted plant virus, *Barley yellow dwarf virus* (BYDV), but used the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), as the vector. This time, either acquisition or inoculation behaviors were terminated by pulling the aphid away from the plant at a specific time point. Results showed that when waveform E2 lasted for 10 min or longer, the acquisition efficiency was 58% and had the strongest relation to virus acquisition, whereas the duration and numbers of waveform E1 showed relevance for virus inoculation (Prado & Tjallingii 1994), similar to X waves in Scheller & Shukle (1986). When combined, the results of Scheller & Shukle (1986) and Prado & Tjallingii (1994) demonstrate that repetitive sieve element punctures (i.e., R-pd's in X waves) and continuous watery salivation (i.e., waveform E1) both represent the complicated behavior prior to acceptance of a sieve element cell, as described above under 'X waves.'

In a study using *Aphis fabae* Scopoli, the average time for aphids to access phloem was 45 min per probe, but the average time to achieve sustained phloem ingestion usually took 3–4 h (Tjallingii 1994). An intriguing relationship was found between waveform E1 and host plant acceptance. Aphids that feed on host plants with varying degrees of resistance usually perform longer durations of E1 events, and sometimes shorter or absent events of E2 (Nielson & Don 1974; Niassy *et al.* 1987; Ryan *et al.* 1987; Dixon *et al.* 1990). This indicates the importance (and potential mechanisms) of salivation against host plant defenses responses and any resistance factors located in phloem sap.

Xylem phase: G. Waveform G is produced occasionally by aphids on diets and plants, and is presumed to be xylem ingestion by histological evidence, which correlated the waveform and the location of stylets in xylem. In addition, starving the aphid or putting aphids in a desiccated environment before the experiment can significantly increase the numbers and durations of waveform G events (Spiller *et al.* 1990). The differences between waveform G and E2 are as follows: (1) more sinusoidal shape, (2) higher amplitude, (3) higher potential level (waveform G has an

extracellular potential level), and (4) more irregular pattern (Tjallingii 1987). As major phloem-ingesters, most of the aphids used in EPG studies didn't show much xylem ingesting. However, alate (winged) *A. fabae* tend to ingest from xylem after they settle on plants (about 60% of individuals), whereas no apterous individuals performed waveform G (Powell & Hardie 2002). The authors concluded that there could be an evolutionary benefit of xylem ingestion for aphid dispersal by different morphological groups of aphids.

APPLICATIONS OF APHID EPG TO IPM RESEARCH

In addition to basic feeding behavior research, the behavioral characteristics of aphids uncovered by EPG can be applied across a wide discipline of practical studies. One of the major applications of EPG is in evaluation of host plant resistance. Aphid's behavioral characteristics on aphid-susceptible and -resistant cultivars or host and non-host plants provides the researcher a time-saving tool to evaluate the degree of resistance (Nielson & Don 1974; Lohar & Kawada 1987; Peters *et al*. 1988; Montllor & Tjallingii 1989; Annan *et al*. 2000; Wang *et al*. 2004), or to identify the location of resistance (van Helden & Tjallingii 1993; Caillaud *et al*. 1995; Kaloshian *et al*. 2000; Zehnder 2001; Sandanayaka *et al*. 2003; Le Roux *et al*. 2008; Lightle *et al*. 2012; Koch *et al*. 2015). Another agricultural application of EPG is evaluation of feeding behavioral changes by the application of pesticides or repellents (Holbrook 1977; Reuter *et al*. 1993; Harrewijn & Kayser 1997; Abraham & Epperlein 1999; Kang *et al*. 2012). Aphids are major vectors of many plant pathogenic viruses that cause enormous loss worldwide. The viral transmission behaviors of aphids have been extensively studied via EPG. Aphid behaviors that are correlated with viral acquisition or inoculation can be identified (Hodges & McLean 1969; Holbrook 1978; Powell 1991; Prado & Tjallingii 1994; Martin *et al*. 1997), and differences in feeding behavior of viruliferous/nonviruliferous aphids or on virus-infected/virus-free host plants can be disclosed (Ullman *et al*. 1988; Blua & Perring 1992; Quiroz *et al*. 1992; Boquel *et al*. 2011, 2012; Hu *et al*. 2013).

EPG can also be applied to studies of ecology and evolutionary biology. The results of interactions between aphids and plants can often be quickly inferred by EPG, which has been mentioned in previous paragraph. In addition, intraspecific interactions or interspecific interactions of aphids were studied by comparing behaviors recorded using EPG monitors (Chongrattanameteekul *et al*. 1991; Formusoh *et al*. 1992; Prado & Tjallingii 1997; Morris & Foster 2008; Brunissen *et al*. 2009). Moreover, behavioral variations were used to reflect genetic variations and to test hypothesis of host specialization, diversification, and sympatric/allopatric speciation of aphids (Caillaud 1999; Powell & Hardie 2000; Funk & Bernays 2001; Tosh *et al*. 2001, 2003; Halarewicz & Gabryś 2012; Kordan *et al*. 2012).

PROSPECTS FOR APHID EPG RESEARCH IN TAIWAN

Although EPG has been used for aphid studies for over 50 years, until recently, there was no research in Taiwan that involved EPG. Aphids are among the most important pests in Taiwan. They can cause severe yield loss by direct feeding damage or transmitting plant viruses (Lee 1986; Bau *et al*. 2004; Deng 2011). There are seven aphid-transmitted plant viruses of economic importance in Taiwan, i.e., *Cucumber mosaic virus*, *Papaya ringspot virus* W strain, *Papaya ringspot virus*, *Potato virus* Y, *Turnip mosaic virus*, *Zucchini yellow mosaic virus*, and *Banana bunchy-top virus* (Deng 2011; Tsai & Chen 2011). Researchers have been struggling for years to find ways to control these viral diseases. One of the most effective and fundamental ways of dealing with viral diseases is breeding resistant strains of

crop plants (Niassy *et al*. 1987; Li *et al*. 2007; Smith & Boyko 2007). Crops resistant to either insect vector or virus can benefit agricultural production by decreasing the transmission of virus; those resistance traits are sometime mutually inclusive (Chen *et al*. 1996). EPG can provide a rapid preliminary evaluation of the crops for aphid resistance in most of the aphid-plant combinations, due to the similarity of waveforms among aphids. Most of the aphid-transmitted viral diseases in Taiwan are non-persistently transmitted, which means the acquisition or inoculation can take place early in a single probe, most likely during pd's in pathway phase. In this case, future EPG research would need to focus on resistance factors located in the epidermis or on the surface of plant that can prevent the transmission behavior.

EPG can also be valuable for study of residual pesticide effects, enabling researchers to evaluate the potential of pesticides for viral disease control. Despite the fact that some of the major aphid vectors in Taiwan have already been studied, the effects of viruses on behaviors of aphids have not been quantified using EPG. EPG is a promising method for researcher to decipher the interaction between aphid and specific virus, and might lead to influential discoveries in basic biology and beneficial application in agriculture.

The above-mentioned examples clearly show the potential and importance of EPG in understanding the behaviors of aphid pests in Taiwan, and it is certain that valuable information will continuously be discovered by the use of EPG.

REFERENCES

Abraham, K. and K. Epperlein. 1999. Influence of imidacloprid after seed-treatment of maize on the sucking behavior of the bird-cherry aphid (*Rhopalosiphum padi* L.) and on the transmission of BYDV using electrical penetration graph technique in laboratory investigations. Gesunde Pflanzen 51:90–94.

Almeida, R. P. P. and E. A. Backus. 2004. Stylet penetration behaviors of *Graphocephala atropunctata* (Signoret) (Hemiptera, Cicadellidae): EPG waveform characterization and quantification. Ann. Entomol. Soc. Amer. 97:838–851.

Almeida, R. P. P. and A. H. Purcell. 2006. Patterns of *Xylella fastidiosa* colonization on the precibarium of sharpshooter vectors relative to transmission to plants. Ann. Entomol. Soc. Amer. 99:884–890.

Annan, I. B., W. M. Tingey, G. A. Schaefers, W. F. Tjallngil, E. A. Backus, and K. N. Saxena. 2000. Stylet penetration activities by *Aphis craccivora* (Homoptera: Aphididae) on plants and excised plant parts of resistant and susceptible cultivars of cowpea (Leguminosae). Ann. Entomol. Soc. Amer. 93:133–140.

Backus, E. A. 1994. History, development, and applications of the AC electronic monitoring system for insect feeding. p.1–51. *in*: History, Development, and Application of AC Electronic Insect Feeding Monitors. (Ellsbury, M. M., E. A. Backus, and D. L. Ullman, eds.) Entomological Society of America. Lanham, MD. 128 pp.

Backus, E. A. Sharpshooter feeding behavior in relation to transmission of *Xylella fastidiosa*: A model for foregut-borne transmission mechanisms. *in*: Vector-Mediated Transmission of Plant Pathogens. (Brown, J. K., ed.) Amer. Phytopathol. Soc. (in press)

Backus, E. A. and W. H. Bennett. 2009. The AC-DC Correlation Monitor: New EPG design with flexible input resistors to detect both R and emf components for any piercing-sucking hemipteran. J. Insect Physiol. 55:869–884.

Backus, E. A., M. J. Devaney, and W. H. Bennett. 2000. Comparison of signal processing circuits among seven AC electronic monitoring systems for their effects on the emf and R components of aphid (Homoptera: Aphididae) waveforms, p.102–143. *in*: Principles and Applications of Electronic Monitoring and Ohter Techniques in the Study of Homopteran Feeding Behavior. (Walker, G. P. and E. A. Backus, eds.) Entomological Society of America. Lanham, MD. 260 pp.

Backus, E. A., J. Habibi, F. Yan, and M. Ellersieck. 2005. Stylet penetration by adult *Homalodisca coagulata* on grape: Electrical penetration graph waveform characterization, tissue correlation, and possible implications for transmission of *Xylella fastidiosa*. Ann. Entomol. Soc. Amer. 98:787–813.

Backus, E. A., W. J. Holmes, F. Schreiber, B. J. Reardon, and G. P. Walker. 2009. Sharpshooter X wave: Correlation of an electrical penetration graph waveform with xylem penetration supports a hypothesized

mechanism for *Xylella fastidiosa* inoculation. Ann. Entomol. Soc. Amer. 102:847–867.

Backus, E. A., M. Rangasamy, M. Stamm, H. J. McAuslane, and R. Cherry. 2013. Waveform library for chinch bugs (Hemiptera: Heteroptera: Blissidae): Characterization of electrical penetration graph waveforms at multiple input impedances. Ann. Entomol. Soc. Amer. 106:524–539.

Backus, E. A., H. J. Shugart, E. E. Rogers, J. K. Morgan, and R. Shatters. 2015. Direct evidence of egestion and salivation of *Xylella fastidiosa* suggests sharpshooters can be "flying syringes". Phytopathology 105:608–620.

Bau, H. J., Y. H. Cheng, T. A. Yu, J. S. Yang, P. C. Liou, C. H. Hsiao, C. Y. Lin, and S. D. Yeh. 2004. Field evaluation of transgenic papaya lines carrying the coat protein gene of *Papaya ringspot virus* in Taiwan. Plant Dis. 88:594–599.

Blua, M. J. and T. M. Perring. 1992. Effects of zucchini yellow mosaic virus on colonization and feeding behavior of *Aphis gossypii* (Homoptera: Aphididae) alatae. Environ. Entomol. 21:578–585.

Boquel, S., C. Delayen, A. Couty, P. Giordanengo, and A. Ameline. 2012. Modulation of aphid vector activity by potato virus y on in vitro potato plants. Plant Dis. 96:82–86.

Boquel, S., P. Giordanengo, and A. Ameline. 2011. Divergent effects of PVY-infected potato plant on aphids. Eur. J. Plant Pathol. 129:507–510.

Brunissen, L., A. Cherqui, Y. Pelletier, C. Vincent, and P. Giordanengo. 2009. Host-plant mediated interactions between two aphid species. Entomol. Exp. Appl. 132:30–38.

Caillaud, C. M. 1999. Behavioural correlates of genetic divergence due to host specialization in the pea aphid, *Acrythosiphon pisum*. Entomol. Exp. Appl. 91:227–232.

Caillaud, C. M., J. S. Pierre, B. Chaubet, and J. P. Di Pietro. 1995. Analysis of wheat resistance to the cereal aphid *Sitobion avenae* using electrical penetration graphs and flow charts combined with correspondence analysis. Entomol. Exp. Appl. 75:9–18.

Chen, J. Q., B. Delobel, Y. Rahbé, and N. Sauvion. 1996. Biological and chemical characteristics of a genetic resistance of melon to the melon aphid. Entomol. Exp. Appl. 80:250–253.

Chongrattanameteekul, W., J. E. Foster, R. H. Shukle, and J. E. Araya. 1991. Feeding behavior of *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.) (Hom., Aphididae) on wheat as affected by conspecific and interspecific interactions. J. Appl. Entomol. 111:361–364.

Deng, T. C. 2011. Evolutionary change of trends in prevalence of cucurbits-infecting viruses in Taiwan, 1981–2011. p.147–163. *in*: Proceedings of the Symposium on Integrated Management Technology of Insect Vectors and Insect-Borne Diseases. July 15, 2011. Taichung, Taiwan. Taiwan Agriculture Research Insttitute, Taichung. (in Chinese with English abstract)

Dixon, A. G. O., P. J. Bramel-Cox, and J. C. Reese. 1990. Feeding behavior of biotype E greenbug (Homoptera: Aphididae) and its relationship to resistance in sorghum. J. Econ. Entomol. 83:241–246.

Dugravot, S., E. A. Backus, B. J. Reardon, and T. A. Miller. 2008. Correlations of cibarial muscle activities of *Homalodisca* spp. sharpshooters (Hemiptera: Cicadellidae) with EPG ingestion waveform and excretion. J. Insect Physiol. 54:1467–1478.

Formusoh, E. S., G. E. Wilde, and J. C. Reese. 1992. Reproduction and feeding behavior of greenbug biotype E (Homoptera: Aphididae) on wheat previously fed upon by aphids. J. Econ. Entomol. 85:789–793.

Funk, D. J. and E. A. Bernays. 2001. Geographic variation in host specificity reveals host range evolution in *Uroleucon ambrosiae* aphids. Ecology 82:726–739.

Halarewicz, A. and B. Gabryś. 2012. Did the evolutionary transition of aphids from angiosperm to non-spermatophyte vascular plants have any effect on probing behaviour? Bull. Insectol. 65:77–80.

Harrewijn, P. and H. Kayser. 1997. Pymetrozine, a fast-acting and selective inhibitor of aphid feeding. In-situ studies with electronic monitoring of feeding behaviour. Pestic. Sci. 49:130–140.

Harris, K. F. and L. J. Harris. 2001. Ingestion-egestion theory of cuticula-borne virus transmission. p. 111–132. *in*: Virus-Insect-Plant Interactions. (Harris, K. F., O. P. Smith, and J. E. Duffus, eds.) Academic Press. San Diego, CA. 376 pp.

Hodges, L. R. and D. L. McLean. 1969. Correlation of transmission of bean yellow mosaic virus with salivation activity of *Acyrthosiphon pisum* (Homoptera: Aphididae). Ann. Entomol. Soc. Amer. 62:1398–1401.

Holbrook, F. R. 1977. Aldicarb and thiofanox: Effect on the feeding activity of green peach aphids. J. Econ. Entomol. 70:742–744.

Holbrook, F. R. 1978. Transmission of potato leaf roll virus by the green peach aphid. Ann. Entomol. Soc. Amer. 71:830–831.

Hu, Z., H. Zhao, and T. Thieme. 2013. Modification of non-vector aphid feeding behavior on virus-infected host plant. J. Insect Sci. 13:1–11.

Jiang, Y. X. and G. P. Walker. 2001. Pathway phase waveform characteristics correlated with length and rate of stylet advancement and partial stylet withdrawal in AC electrical penetration graphs of adult whiteflies. Entomol. Exp. Appl. 101:233–246.

Kaloshian, I., M. G. Kinsey, V. M. Williamson, and D. E. Ullman. 2000. Mi-mediated resistance against the potato aphid *Macrosiphum euphorbiae* (Hemiptera: Aphididae) limits sieve element ingestion. Environ. Entomol. 29:690–695.

Kang, M. A., M. J. Seo, I. C. Hwang, C. Jang, H. J. Park, Y. M. Yu, and Y. N. Youn. 2012. Insecticidal activity and feeding behavior of the green peach aphid, Myzus persicae, after treatment with nano types of pyrifluquinazon. J. Asia Pac. Entomol. 15:533–541.

Kimmins, F. M. and W. F. Tjallingii. 1985. Ultrastructure of sieve element penetration by aphid stylets during electrical recording. Entomol. Exp. Appl. 39:135–141.

Koch, K. G., N. Palmer, M. Stamm, J. D. Bradshaw, E. Blankenship, L. M. Baird, G. Sarath, and T. M. Heng-Moss. 2015. Characterization of greenbug feeding behavior and aphid (Hemiptera: Aphididae) host preference in relation to resistant and susceptible tetraploid switchgrass populations. Bioenerg. Res. 8:165–174.

Kordan, B., K. Dancewicz, A. Wróblewska, and B. Gabryś. 2012. Intraspecific variation in alkaloid profile of four lupine species with implications for the pea aphid probing behaviour. Phytochem. Lett. 5:71–77.

Le Roux, V., S. Dugravot, E. Campan, F. Dubois, C. Vincent, and P. Giordanengo. 2008. Wild *Solanum* resistance to aphids: Antixenosis or antibiosis? J. Econ. Entomol. 101:584–591.

Lee, H. 1986. Seasonal occurrence of the important insect pests on cabbage in southern Taiwan. J. Agric. Res. China 35:530–542. (in Chinese with English abstract)

Leopold, R. A., T. P. Freeman, J. S. Buckner, and D. R. Nelson. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata*, (Homoptera: Cicadellidae). Arthropod Struct. Dev. 32:189–199.

Li, Y., C. B. Hill, S. R. Carlson, B. W. Diers, and G. L. Hartman. 2007. Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breed. 19:25–34.

Lightle, D. M., M. Dossett, E. A. Backus, and J. C. Lee. 2012. Location of the mechanism of resistance to *Amphorophora agathonica* (Hemiptera: Aphididae) in red raspberry. J. Econ. Entomol. 105:1465–1470.

Lohar, M. K. and K. Kawada. 1987. Probing behaviour of the aphid, *Schizaphis graminum* (Rondani), *Rhopalosiphum maidis* (Fitch) and L*ongiunguis sacchari* (Zehntner) on resistant and susceptible sorghum plants. Ber. Ohara Inst. Iandw. Biol., Okayama Univ. 19:137–144.

Martin, B., J. L. Collar, W. F. Tjallingii, and A. Fereres. 1997. Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of nonpersistently transmitted plant viruses. J. Gen. Virol. 78:2701–2705.

McLean, D. L. and M. G. Kinsey. 1964. A technique for electronically recording aphid feeding and salivation. Nature 202:1358–1359.

McLean, D. L. and M. G. Kinsey. 1967. Probing behavior of the pea aphid, *Acyrthosiphon pisum*. I. Definitive correlation of electronically recorded waveforms with aphid probing activities. Ann. Entomol. Soc. Amer. 60:400–405.

McLean, D. L. and W. A. Weigt, Jr. 1968. An electronic measuring system to record aphid salivation and ingestion. Ann. Entomol. Soc. Amer. 61:180–185.

Miranda, M. P., A. Fereres, B. Appezzato-Da-Gloria, and J. R. S. Lopes. 2009. Characterization of electrical penetration graphs of *Bucephalogonia xanthophis*, a vector of *Xylella fastidiosa* in citrus. Entomol. Exp. Appl. 130:35–46.

Montllor, C. B. and W. F. Tjallingii. 1989. Stylet penetration by two aphid species on susceptible and resistant lettuce. Entomol. Exp. Appl. 52:103–111.

Moreno, A., E. Garzo, G. Fernandez-Mata, M. Kassem, M. A. Aranda, and A. Fereres. 2011. Aphids secrete watery saliva into plant tissues from the onset of stylet penetration. Entomol. Exp. Appl. 139:145–153.

Morris, G. and W. A. Foster. 2008. Duelling aphids: Electrical penetration graphs reveal the value of fighting for a feeding site. J. Exp. Biol. 211:1490–1494.

Niassy, A., J. D. Ryan, and D. C. Peters. 1987. Variations in feeding behavior, fecundity, and damage of biotypes B and E of *Schizaphis graminum* (Homoptera: Aphididae) on three wheat genotypes. Environ. Entomol. 16:1163–1168.

Nielson, M. W. and H. Don. 1974. Probing behavior of biotypes of the spotted alfalfa aphid on resistant and susceptible alfalfa clones. Entomol. Exp. Appl. 17:477–486.

Pearson, C. C., E. A. Backus, H. J. Shugart, and J. E. Munyaneza. 2014. Characterization and correlation of epg waveforms of *Bactericera cockerelli* (Hemiptera: Triozidae): Variability in waveform appearance in relation to applied signal. Ann. Entomol. Soc. Amer. 107:650–666.

Peters, D. C., D. Kerns, G. J. Puterka, and R. McNew. 1988. Feeding behavior, development, and damage by biotypes B, C, and E of *Schizaphis graminum* (Homoptera: Aphididae) on 'Wintermalt' and 'Post' barley. Environ. Entomol. 17:503–507.

Pollard, D. G. 1973. Plant penetration by feeding aphids (Hemiptera, Aphidoidea): A review. Bull. Entomol. Res. 62:631–714.

Powell, G. 1991. Cell membrane punctures during epidermal penetrations by aphids: consequences for the transmission of two potyviruses. Ann. Appl. Biol. 119:313–321.

Powell, G. L. E. N. and J. I. M. Hardie. 2000. Host-selection behaviour by genetically identical aphids with different plant preferences. Physiol. Entomol. 25:54–62.

Powell, G. and J. Hardie. 2002. Xylem ingestion by winged aphids. Entomol. Exp. Appl. 104:103–108.

Powell, G., T. Pirone, and J. Hardie. 1995. Aphid stylet activities during potyvirus acquisition from plants and an in vitro system that correlate with subsequent transmission. Eur. J. Plant Pathol. 101:411–420.

Powell, G., C. R. Tosh, and J. Hardie. 2006. Hostplant selection by aphids: Behavioral, evolutionary, and applied perspectives. Annu. Rev. Entomol. 51:309–330.

Prado, E. and W. F. Tjallingii. 1994. Aphid activities during sieve element punctures. Entomol. Exp. Appl. 72:157–165.

Prado, E. and W. F. Tjallingii. 1997. Effects of previous plant infestation on sieve element acceptance by two aphids. Entomol. Exp. Appl. 82:189–200.

Purcell, A. H., A. H. Finlay, and D. L. McLean. 1979. Pierce's disease bacterium: Mechanism of transmission by leafhopper vectors. Science 206:839–841.

Quiroz, C., R. M. Lister, R. H. Shukle, J. E. Araya, and J. E. Foster. 1992. Selection of symptom variants from the NY-MAV strain of barley yellow dwarf virus and their effects on the feeding behavior of the vector *Sitobion avenae* (Homoptera: Aphididae). Environ. Entomol. 21:376–381.

Reese, J. C., D. C. Margolies, E. A. Backus, S. Noyes, P. Bramel-Cox, and A. G. O. Dixon. 1994. Characterization of aphid host plant resistance and feeding behavior through use of a computerized insect feeding monitor. p.52–72. *in*: History, Development, and Application of AC Electronic Insect Feeding Monitors. (Ellsbury, M. M., E. A. Backus, and D. L. Ullman eds.) Entomological Society of America. Lanham, MD. 128 pp.

Reese, J. C., W. F. Tjallingii, M. Van Helden, and E. Prado. 2000. Waveform comparisons among AC and DC electronic monitoring systems for aphid (Homoptera: Aphididae) feeding behavior. p.70–101. *in*: Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior. (Walker, G. P. and E. A. Backus, eds.) Entomological Society of America. Lanham, MD. 260 pp.

Reuter, L. L., N. C. Toscano, and T. M. Perring. 1993. Modification of feeding behavior of *Myzus persicae* (Homoptera: Aphididae) by selected compounds. Environ. Entomol. 22:915–919.

Ryan, J. D., K. W. Dorschner, M. Girma, R. C. Johnson, and R. D. Eikenbary. 1987. Feeding behavior, fecundity, and honeydew production of two biotypes of greenbug (Homoptera: Aphididae) on resistant and susceptible wheat. Environ. Entomol. 16:757–763.

Sandanayaka, W. R. M., V. G. M. Bus, P. Connolly, and R. Newcomb. 2003. Characteristics associated with woolly apple aphid *Eriosoma lanigerum*, resistance of three apple rootstocks. Entomol. Exp. Appl. 109:63–72.

Schaefers, G. A. 1966. The use of direct current for electronically recording aphid feeding and salivation. Ann. Entomol. Soc. Amer. 59:1022–1024.

Scheller, H. V. and R. H. Shukle. 1986. Feeding behavior and transmission of barley yellow dwarf virus by *Sitobion avenae* on oats. Entomol. Exp. Appl. 40:189–195.

Smith, C. M. and E. V. Boyko. 2007. The molecular bases of plant resistance and defense responses to aphid feeding: Current status. Entomol. Exp. Appl. 122:1–16.

Spiller, N. J., L. Koenders, and W. F. Tjallingii. 1990. Xylem ingestion by aphids: A strategy for maintaining water balance. Entomol. Exp. Appl. 55:101–104.

Sylvester, E. S. 1980. Circulative and propagative virus transmission by aphids. Annu. Rev. Entomol. 25:257–286.

Tjallingii, W. F. 1978. Electronic recording of penetration behaviour by aphids. Entomol. Exp. Appl. 24:721–730.

Tjallingii, W. F. 1982. Electrical recording of aphid penetration. p.409–410. *in*: Proceedings of the 5th International Symposium on Insect-Plant Relationships. March 1–4, 1982. Centre for Agricultural Publication and Documentation, Wageningen, The Netherlands.

Tjallingii, W. F. 1985a. Electrical nature of recorded signals during stylet penetration by aphids. Entomol. Exp. Appl. 38:177–186.

Tjallingii, W. F. 1985b. Membrane potentials as an indication for plant cell penetration by aphid stylets. Entomol. Exp. Appl. 38:187–193.

Tjallingii, W. F. 1987. Stylet penetration activities by aphids: New correlations with electrical penetration graphs. p.301–306. *in*: Proceedings of the 6th International Symposium on Insect-Plant Relationships. May 31, 1987. Junk. Dordrecht, Germany.

Tjallingii, W. F. 1990. Continuous recording of stylet penetration activities by aphids. p.89–99. *in*: Aphid-plant Genotype Interactions. (Campbell, R. K. and R. D. Eikenbary, eds.) Elsevier Science Pub. Amsterdam, The Netherlands. 378 pp.

Tjallingii, W. F. 1994. Sieve element acceptance by aphids. Eur. J. Entomol. 91:47–52.

Tjallingii, W. F. 2000. Comparison of AC and DC systems for electronic monitoring of stylet penetration activities by homopterans. p.41–69. *in*: Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior. (Walker, G. P. and E. A. Backus, eds.) Entomological Society of America. Lanham, MD. 260 pp.

Tjallingii, W. F. and B. Gabryś. 1999. Anomalous stylet punctures of phloem sieve elements by aphids. Entomol. Exp. Appl. 91:97–103.

Tjallingii, W. F. and T. Hogen Esch. 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiol. Entomol. 18:317–328.

Tosh, C. R., G. Powell, and J. Hardie. 2003. Decision making by generalist and specialist aphids with the same genotype. J. Insect Physiol. 49:659–669.

Tosh, C. R., K. F. A. Walters, and A. E. Douglas. 2001. On the mechanistic basis of plant affiliation in the black bean aphid (*Aphis fabae*) species complex. Entomol. Exp. Appl. 99:121–125.

Tsai, C. W. and W. T. Chen. 2011. Control strategies of important aphids that transmit viral diseases of crops. p.183–191. *in*: Proceedings of the Symposium on Integrated Management Technology of Insect Vectors and Insect-Borne Diseases. July 1–2, 2011. Taichung, Taiwan. Taiwan Agriculture . Research Institute, Taichung. (in Chinese with English abstract)

Ullman, D. E., C. O. Qualset, and D. L. McLean. 1988. Feeding responses of *Rhopalosiphum padi* (Homoptera: Aphidae) to barley yellow dwarf virus resistant and susceptible barley varieties. Environ. Entomol. 17:988–991.

van Helden, M. and W. F. Tjallingii. 1993. Tissue localisation of lettuce resistance to the aphid *Nasonovia ribisnigri* using electrical penetration graphs. Entomol. Exp. Appl. 68:269–278.

Walker, G. P. 2000. A beginner's guide to electronic monitoring of homopteran probing behavior. p.14–40. *in*: Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior. (Walker, G. P. and E. A. Backus, eds.) Entomological Society of America. Lanham, MD. 260 pp.

Wang, Y. M., P. F. Zhang, and J. Q. Chen. 2004. Host-preference biotypes of the cotton aphid, *Aphis gossypii* glover and the behavioral mechanism in their formation. Acta Entomol. Sin. 47:760–767.

Zehnder, G. W., A. J. Nichols, O. R. Edwards, and T. J. Ridsdill-Smith. 2001. Electronically monitored cowpea aphid feeding behavior on resistant and susceptible lupins. Entomol. Exp. Appl. 98:259–269.

昆蟲刺探電位圖譜 (EPG)：刺吸式害蟲取食行為鑑定新技術 †

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摘要

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由於無法直接觀察半翅目昆蟲特化口針在植物組織的刺探與穿刺過程，致使研究此類昆蟲的取食行為、對植物損害以及傳播植物病原的相關研究，存在極大挑戰，及至 50 年前所發明昆蟲刺探電位圖譜 (electrical penetration graph; EPG) 監測儀，才克服這些研究上的障礙。如今 EPG 主要以 3 個方向發展半翅目害蟲整合管理 (IPM) 新策略，包括 (1) 對特定半翅目昆蟲之取食行為與造成植物傷害機制未明時，EPG 是可有效解決的工具；(2) 解決上述問題之後，EPG 可被應用於分析殺蟲劑或拒食劑對害蟲行為所造成的影響，並進一步評估其他化合物對於特定取食行為造成危害與傳病的原因；(3) EPG 能被應用於不同植物品系抗蟲程度的評估，包括基因改造表現生物殺蟲成分的作物。本回顧報告主要目的包括 (1) EPG 的原理以及歷史，特別針對第 3 代 AC-DC 儀器進行介紹；(2) 以蚜蟲為模式系統，作為瞭解那些波形可自 EPG 獲得；(3) EPG 可應用在台灣農業的那些需求，特別是對於那些具有經濟重要性的蚜蟲。本文係首次提出 3 型 EPG 在蚜蟲研究概況的回顧研究報告。

關鍵詞：昆蟲刺探電位圖譜、半翅目、取食行為。

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DOI: 10.6156/JTAR/2016.06503.02 **專題論述**

叢枝菌根菌分類學：繡球菌門之最新定義

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摘要

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本文主要就目前叢枝菌根菌歸屬於繡球菌門之最新分類觀念與各屬之最新定義提出說明，並就目前台灣工業界已生產之叢枝菌根菌或田間試驗所使用之菌種名稱，各按新屬之定義重新調整。本文也提供了繡球菌門以下，各科各目及各屬間的檢索表供研究者參考。

關鍵詞：叢枝菌根、繡球菌門、檢索表。

前言

叢枝菌根菌 (arbuscular mycorrhizal fungi) 的研究，若從 1976 年 Dr. James Trappe 訪台引進菌根觀念與技術迄今，已逾 30 年。這期間我國的菌根研究在國立台灣大學森林系胡弘道教授、植物系陳瑞青教授、園藝系張喜寧教授、植物病理系曾顯雄教授等人及其弟子們的積極推動下，我國在菌根方面的研究曾有過蓬勃發展的階段。但近年來許多與菌根相關的教授們紛紛退休或年紀老邁，再加上欠缺政府的研究經費支持下，我國在菌根方面的研究可能因動能不足而逐漸式微。

由於分子生物學技術在生物領域的研究扮演著舉足輕重的腳色，藉由核醣體 DNA 序列 (rDNA sequence) 來判定親緣關係的技術，已廣泛應用在叢枝菌根菌的分類上。近 10 年來，隨著叢枝菌根菌培養技術的進步以及 rDNA 資料庫 (http://www.ncbi.nlm.nih.gov) 的建立與序列 (圖 1) 的比對，叢枝菌根菌的分類觀念與過去相較，已有顯著的差異。為此，筆者擬將目前叢枝菌根菌在分類上的觀念重新整理，冀望能拋磚引玉來引起台灣菌根學界的注意，重新點燃菌根研究的火苗。

過去叢枝菌根菌的分類常受制於繁殖技術等諸多門檻，沒有機會充分瞭解該類真菌的特徵，因此大多僅從土壤中所篩取的孢子或孢子果 (sporocarp) 等野外材料，進行形態特徵的描述。由於無法瞭解孢子的形成過程，常有瞎子摸象的情形發生。過去就曾發生同一菌種，以 2 種新種命名，甚至同時出現在同一期刊的尷尬 (例如：*Glomus leptotichum*, *Glomus fecundisporum*) (Schenck & Smith 1982)。*Acaulospora gerdemannii* 在 1979 年首度被命名之後 (Nicolson & Schenck 1979)，在 1984 年又被以 *Acaulospora appendicula* 重新命名 (Schenck *et al*. 1984)。更有趣的是，經由盆栽培養後，發現先前發表過的菌種 *A. gerdemannii* 竟然與 *G. leptotichum* 是同一菌種，也就是說同一種菌根菌卻可以形成 2 種不同的孢子型態。目前這種可產生 2 種類型孢子的叢枝菌根菌，已被重新歸類在 *Ambispora* 屬中 (Walker *et al*. 2007)。另外，Wu *et al*. (1995) 以新種發表 *Glomus chimonobambusae* 時，曾在論文中指出本菌種的內壁可與梅澤試劑 (Melzer's reagent) 反應 (染成粉紅色) 且沒有固定厚度 (amorphous)；以及發芽時，發芽管是自孢

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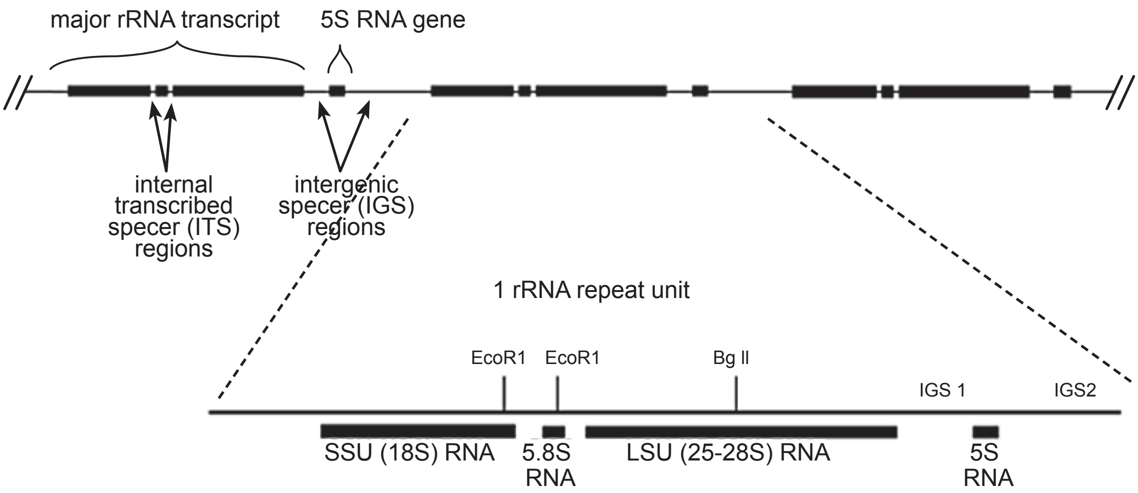

圖 1. 小次元體 (SSU)、大次元體 (LSU)、ITS (內部轉錄間隔)、IGS (間隔基因) 在 rDNA 的相對位置。

Fig. 1. The nuclear-encoded ribosomal RNA genes (rDNA) of fungi exist as a multiple-copy gene family comprised of small subunit (SSU), 5.8 S, large subunit (LSU) ribosomal DNA, intergenic spacer (IGS), and 5S ribosomal DNA arranged in a head-to-toe manner (http://sites.biology.duke.edu/fungi/mycolab/primers.htm).

子壁直接穿出，這種特殊發芽方式與傳統的 *Glomus* 屬不同。一般 *Glomus* 屬的真菌發芽時，發芽管是自接著菌絲 (attached hypha) 的管道中長出。後續的研究才發現 *G. chimonobambusae* 雖然形態像 *Glomus*，但是孢子內壁卻與無柄孢子屬 (*Acaulospora*)、盾蓋孢子屬 (*Scutellospora*) 類似，於孢子發芽時，與無柄孢子屬、盾蓋孢子屬一樣，會形成發芽包 (germination orb) 或發芽盾 (germ shield)。目前這類真菌因具有繡球孢子的外型，但卻擁有與無柄孢子屬、盾蓋孢子屬等類似的孢壁與發芽特徵，因此已被重新分類在 *Pacispora* 屬之下 (Oehl & Sieverding 2004)。

在過去的分類觀念，較強調以形態特徵作為屬的分類依據，例如：孢子有無菌絲柄 (attached hypha)、孢子發芽有無形成發芽盾、產孢的方式、孢子在產孢菌絲的相對位置等，但隨著時間與菌根知識的累積，科學家們已經瞭解到，過去作為屬的主要分類特徵，如今已不再適用。目前的 rDNA 序列的比對，解釋了許多叢枝菌根菌分類上的矛盾，同時也提供我們對叢枝菌根菌分類一個更合乎自然演化法則與親緣關係的瞭解。雖然現在叢枝菌根菌的分類是以分子生物學為基礎進行研究，但仍然脫離不了對該菌的培養與形態的充分認識，這也是筆者寫下這篇文章的最大動機所在。本文將提供繡球菌門分類項下的所有各子單元之說明與雙叉檢索表 (附錄)，以利讀者檢索參考。

繡球菌門 (Glomeromycota) (Schüßler *et al*. 2001)

繡球菌門 (Schüßler *et al*. 2001) 這類真菌普遍被認為是絕對共生的；但也有例外，根據 Hildebrandt *et al*. (2002) 的報導，*Glomus intraradices* 可以在沒有宿主植物的情形下完成產孢。 截至目前為止，只有一屬的叢枝菌根菌 (*Geosiphon*) 與藍綠藻 (*Nostoc*) 共生 (Schüßler 2002)，其餘皆與一般有胚植物 (embryophyte) 共生。除了陸地的維管束植物外，苔癬類植物也可形成叢枝菌根。許多本門的真菌已確定可形成叢枝菌根，但也有許多只是從野外採集的材料中分離出孢子而已，其是否可形成叢枝菌根，則尚未可知。有些種迄今仍可培養在盆栽內，因此可提供活材料進行 DNA 分析；但有些則因研究人員的異動或其他原因而失去活性，無法提供 DNA 材料。若是來自野外的材料很多，則有機會採到足量的 DNA 材料進行分析；但仍有許多的種，因無法採得

DNA 材料，以致於真正的演化親緣關係，至今仍無法確定。

本門的真菌在過去，大多數是根據產自土壤或宿主 (host) 根內的孢子之形態來進行描述與命名。本門的真菌菌絲大多沒有隔板。形成孢子的方式，大多是在菌絲的末端膨大後，孢壁逐漸加厚累積而形成。孢子有時以隔板，孢壁直接加厚，或直接在孢子柄的空腔中沉積阻塞物來分隔孢子與其產孢菌絲。有些較複雜的孢子則是形成於膨大的囊袋 (saccule) 內或是形成於囊袋的柄內或柄的外側等。厚壁孢子產生於土壤中或偶而在地面上，可能是單一孢子，疏鬆的孢子堆或是緊密沒有包被 (peridium) 的孢子堆，也可能是具有包被包裹的孢子果。這些孢子果產生在土壤表層上，或接近土壤表層的土壤中或在植物根內。大小在 40–800 μm，具有多層孢壁，含有上百個至上千個細胞核。本門含 1 個繡球菌綱 (Glomeromycetes) (Cavalier-Smith 1998)，4 個目：古生孢子菌目 (Archaeosporales)、類繡球菌目 (Paraglomerales)、繡球菌目 (Glomerales) 與多型孢子菌目 (Diversisporales) (圖 2) (Schüßler *et al*. 2001)。古生孢子菌目下分 3 個科 (Geosiphonaceae、Ambisporaceae、Archaeosporaceae)。類繡球菌目下有 1 個科 (Paraglomeraceae)。繡球菌目下則有 2 個科 (Glomeraceae 與 Claroideoglomeraceae)。多型孢子菌目下有 4 個科 (Gigasporaceae、Acaulosporaceae、Pacisporaceae 與 Diversisporaceae) (表 1)。

就整個演化樹 (圖 2) 來看，類繡球孢子目與古生孢子目應是較為原始的群體。它們都具有孢子無色，菌根染色不易等共同特徵。所產生的孢子以繡球型 (glomoid) 為主，但在 *Archaeospora* 和 *Ambispora* 兩者皆可產生類無柄孢子 (acaulosporoid)。只是這些類無柄孢子的內壁並不與梅澤試劑起反應 (無法染上顏色)。*Archaeospora* 的孢子似乎為演化成無柄孢子屬與內柄生孢子屬的過渡階段，目前本屬有 2 個種 (*Archaeospora trappei*, *Archaeospora schenckii*)，依其形態特徵曾分別被命名為 *Acaulospora trappei* 和 *Entrophospora schenckii*。內柄生孢子屬雖然目前暫時與無柄孢子屬合併，但 *Entrophospora infrequens* 的定位尚未明確，有待後續更多的菌種材料加入分析後方能確定。

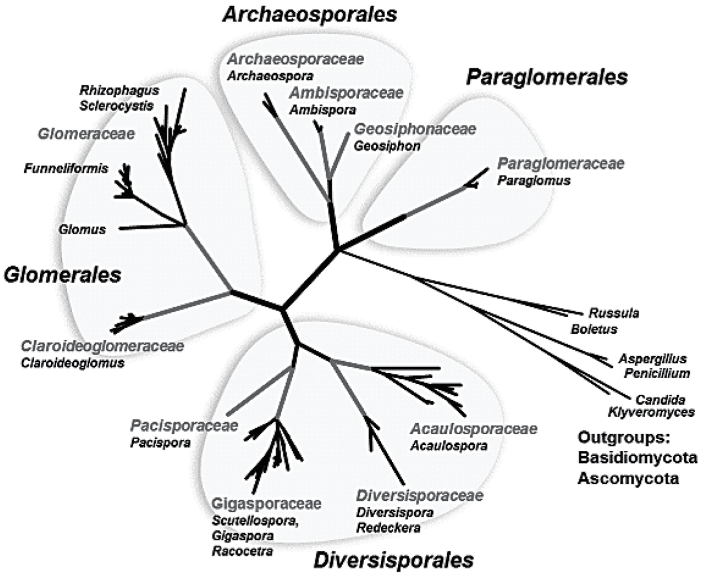

圖 2. 繡球菌門所屬 4 個目及各科間的關係。

Fig. 2. Four orders of Glomeromycota and their inter-relationships (http://schuessler.userweb.mwn.de/geosiphon/geosiphon.html).

表 1. 目前普遍被接受的繡球菌門 (Glomeromycota) 分類單元。

Table 1. The most recent classification of Glomeromycota and its taxa[z].

Phylum *Glomeromycota*		
Class *Glomeromycetes*		
Order (4)	Family (10)	Genus (17)
Glomerales	*Glomeraceae*	*Glomus* *Funneliformis* *Rhizophagus* *Sclerocystis* *Septoglomus*
	Claroideoglomeraceae	*Claroideoglomus*
Diversisporales	*Gigasporaceae*	*Gigaspora* *Racocetra* *Cetraspora* *Dentiscutata* *Scutellospora*
	Acaulosporaceae	*Acaulospora*
	Pacisporaceae	*Pacispora*
	Diversisporaceae	*Diversispora* *Redeckera*
Archaeosporales	*Geosiphonaceae*	*Geosiphon*
	Ambisporaceae	*Ambispora*
	Archaeosporaceae	*Archaeospora*
Paraglomerales	*Paraglomeraceae*	*Paraglomus*

[z] Redecker *et al*. (2013); INVAM (http://invam.wvu.edu/the-fungi/classification).

繡球菌目 (Glomerales) (Morton & Benny 1990)

本目之菌種大多數產孢於地下，有時在土壤表層；可與植物形成內生菌根或類共生菌根，產生孢子、囊泡 (vesicle) 或叢枝體 (arbuscule)；營養菌絲大多沒有隔板，但在菌絲受傷為避免細胞質流失或產生休眠孢子時會形成隔板；無性生殖於菌絲末端或有時在菌絲間形成孢子；孢子單一或形成孢子堆或孢子果；本目與其他形成叢枝菌根的真菌鑑別，主要是根據其 rRNA 的小次元體 (SSU) 基因的標誌序列 YTRRY/2-5/RYYARGTYGN-CARCTTCTTAGAGGGACTATCGGTGTYTA-ACCGRTGG。

本序列與酵母菌的同源位置 (homologous position) 1353 相當 (*Saccharomyces cerevisiae* SSU rRNA sequence J01353)。下標線所示核甘酸序列為繡球菌目特有。上述標誌序列中的數字/(2-5)/代表鹼基有缺失的數目。

繡球菌科 (Glomeraceae) (Pirozynski & Dalpé 1989; Walker & Schüßler 2004)

本科孢子繡球型，產生於地表或接近地表的土壤中；產生單一孢子、或形成孢子堆、或產生於具有包被或沒有包被的孢子果中；其 rRNA 小次元體 (SSU) 基因的標誌序列為 TGTYADGGCAYYRCACYGG。菌根的特徵為胞內叢枝體一般可被酸性染劑如錐蟲藍 (trypan blue) 染成深藍色。根內囊泡是否會形成，取決於叢枝菌根真菌的種類與形成菌根時土壤環境的變化等因素。當大量形成囊泡時，囊泡可能集中於侵入點附近，但也可能廣泛分散於菌根內。這些囊泡大多呈長橢圓形，且被染劑染成深色。在菌根發育階段的後期，或是當田間的根部失去養分吸收能力且逐漸老化的時候，根內會有較多的囊泡出現。根內菌絲在侵入點附近會有菌絲捲或分叉的現象。當菌絲在根內拓殖的時候，常呈互相平行或與根軸平行的方式前進，1.5–4 μm 寬。菌絲與菌絲間

常呈「H」型相互連結，且可被酸性染劑 (如錐蟲藍) 深深著色。根外菌絲常因不同菌種而有不同的數量、分布與形態的差異。無性孢子或單獨產生，或聚集成堆，或產生於雜亂的菌絲團中，或形成於高度秩序化的菌絲團中。孢子壁層層加厚且常與接著菌絲壁 (subtending hypha) 相連。孢子也可形成於根內，可能是由根內囊泡壁加厚後轉化為孢子。大多數的孢子是形成於菌絲的末端，但也有孢子形成於菌絲間的少數特例。例如：*Glomus multicaule*，*Glomus formosanum* (圖 3) (Wu & Chen 1986) 孢子的兩端各有 1 到 2 條接著菌絲。孢子的內含物與接著菌絲的隔離可經由不同的機制來完成，例如：不定型的塞子 (plug)，隔板，層壁 (孢壁的組成之一) 的內層，或直接由層壁逐漸加厚後將接著菌絲的內腔封住等。大多數本科菌種的孢壁多具有黏質的外層，是年輕菌絲壁與年輕孢壁的主要成分，可被梅澤試劑染成粉紅色或紅紫色 (圖 4)。此外，在孢子黏質外壁的下方還有 1 個較堅硬的內層 (或層壁)，這內層與接著菌絲壁相連。在這堅硬的孢壁的內層下方可能還有 1 層具有彈性的薄膜層，當孢子被壓破時，會與堅硬的內層分離。本科的孢子發芽時，其發芽管會自接著菌絲的內腔向外延伸長出 (圖 5)。

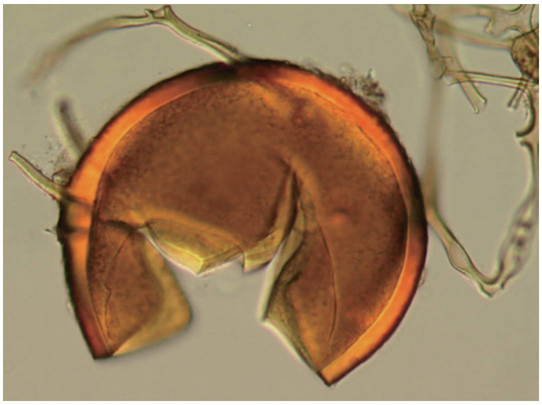

圖 3. 發現於台灣的繡球菌屬新種 *Glomus formosanum*，孢子具有 1–4 枝的接著菌絲。

Fig. 3. *Glomus formosanum*, a new species originally recovered in Taiwan. Note its spore with 1–4 attached hyphae (Scale bar = 15 μm).

繡球孢子屬 (*Glomus*) (Tulasne & C. Tulasne 1845; Walker & Schüßler 2004)

孢子繡球狀；產生於地表或接近於地表的土壤中；可能產生於具有包被或被部分菌絲包裹的孢子果中，也可能單獨，或聚集成堆於土壤或其他基質上 (圖 6、圖 7)。其 rRNA

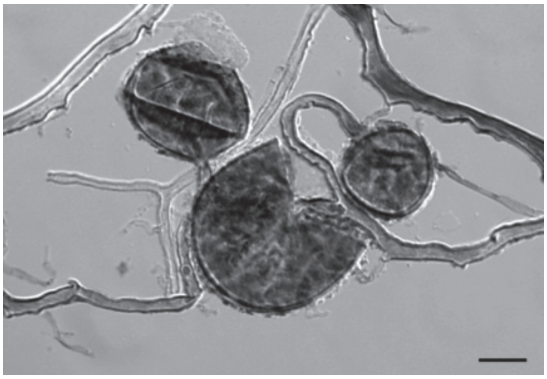

圖 4. *Glomus hoi* 孢子的黏質外層與接著菌絲可被梅澤試劑染成紅紫色。

Fig. 4. The spores of *Glomus hoi*. Note the outer mucilaginous layer of spores and their subtending hyphae stained reddish purple in Melzer's reagent (Scale bar = 40 μm).

圖 5. *Funnelliformis mosseae* 孢子發芽時，雙叉分枝的發芽管自接著菌絲長出。

Fig. 5. The spore germination of *Funnelliformis mosseae*. Note the dichotomous branches of germ tube extending out of attached hypha (Scale bar = 150 μm).

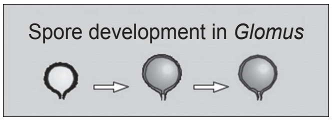

圖 6. 繡球菌屬的產孢過程。

Fig. 6. The spore ontogeny of *Glomus* (http://invam.wvu.edu/the-fungi/classification/glomaceae/glomus).

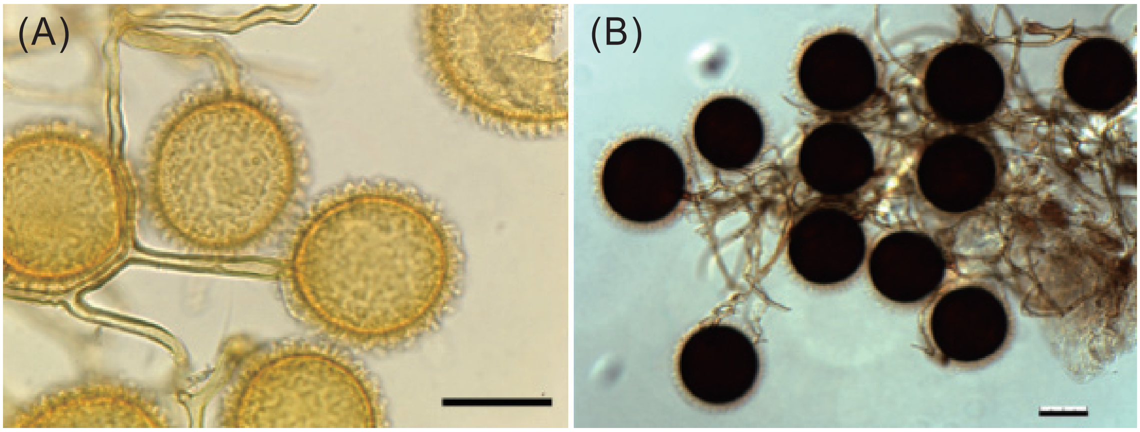

圖 7. *Glomus pansihalos* 常見於花蓮海邊植被的根圈土壤 (A) 及老鼠的腸道 (B) 中。

Fig. 7. *Glomus pansihalos* was frequently found in the (A) rhizosphere of beach plants and in the (B) intestinal tracts of mouse in Hualien area, Taiwan (Scale bar = 100 μm).

次元體 (SSU) 基因的標誌序列為 GGTACGYACTGGTATCATTGG 和 TCGGCTGTAAAAGGCYYTTG。該兩段序列為本屬所特有。

漏斗狀孢子屬 (*Funneliformis*) (Walker & Schüßler 2004)

本屬產生有顏色的孢子；孢子單獨產生於土壤中，或聚集成堆 (1–20 個孢子)，四周有粗菌絲網包裹；孢子基部如漏斗狀，孢壁 2–3 層，外壁透明且隨著孢子的成熟而逐漸脫離，孢子通常在孢子基部的下緣有一隔板；孢子形成的方式與繡球孢子屬同 (圖 8)；其 rRNA 小次元體 (SSU) 基因的標誌序列為 CGGTCATGCCGTTGGTATGY。該段序列為本屬所特有。

根生孢子屬 (*Rhizophagus*) (Walker & Schüßler 2004)

繡球狀孢子單獨產生於土壤中，或成團聚集於土壤或根內；除了囊泡形成 (在侵入點時) 較其他叢枝菌根菌早之外，也形成典型叢枝菌根。孢子形成的方式與繡球孢子屬同。在台灣常見的 *Glomus intraradices*、*Glomus clarum*

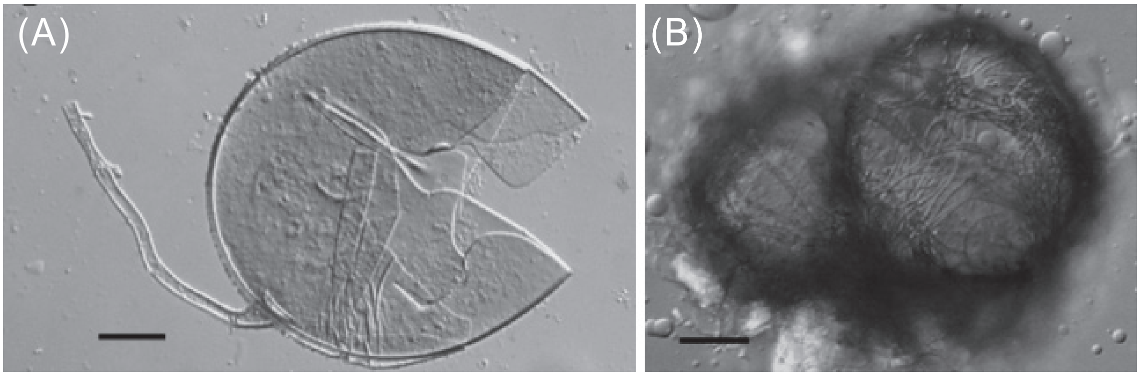

圖 8. (A) 在台灣常見的 *Glomus mosseae* 如今已更名為 *Funneliformis mosseae*。(B) *Funneliformis mosseae* 的孢子果，孢被菌絲可被梅澤試劑染成紅褐色。

Fig. 8. (A) *Glomus mosseae* is very common in Taiwan, and now renamed as *Funneliformis mosseae*. (B) The sporocarp of *F. mosseae*. Note the peridial hyphae stained reddish brown in Melzer's reagent (Scale bar = 50 μm).

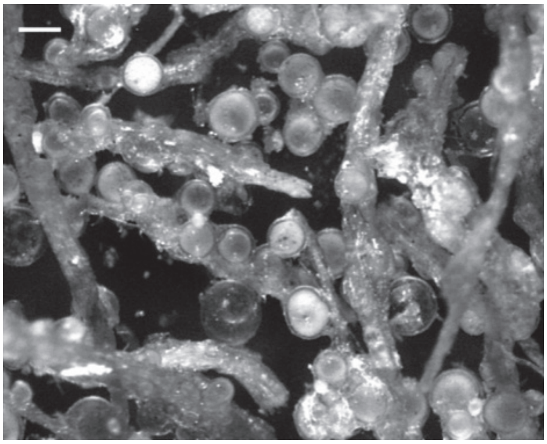

圖 9. 產孢於根內的 *Rhizophagus clarus*。

Fig. 9. The spores of *Rhizophagus clarus* produced within plant roots (Scale bar = 150 μm).

(圖 9) 及 *Glomus manihotis* 如今已分別更名為 *Rhizophagus intraradices*、*Rhizophagus clarus* (圖 9) 及 *Rhizophagus manihotis*。

硬囊孢子屬 (*Sclerocystis*) (Berkeley & Broome 1873)

本屬皆產生孢子果 (圖 10、圖 11)；孢子果為球形、亞球形或半球形；具包被或裸露，具 1 或多枝菌絲柄；厚壁孢子自孢子果中心叢向外形成；孢子與孢子緊緊相鄰成 1 層；中心

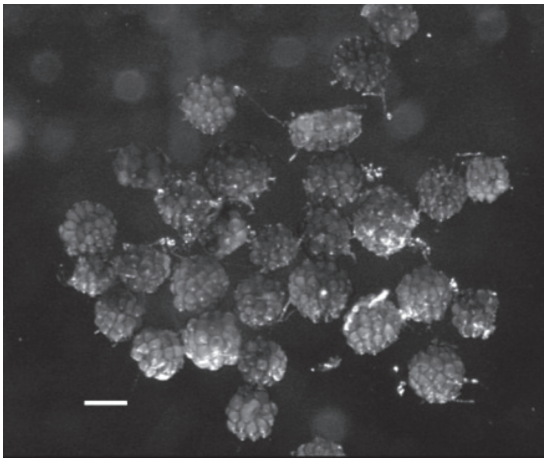

圖 10. *Sclerocystis sinuosa* 的孢子果。

Fig. 10. Sporocarps of *Sclerocystis sinuosa* (Scale bar = 300 μm).

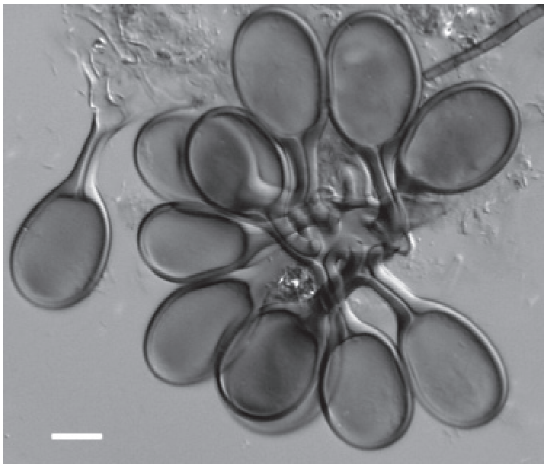

圖 11. *Sclerocystis rubiformis* 的孢子果。

Fig. 11. Sporocarp of *Sclerocystis rubformis* (Scale bar = 20 μm).

叢菌絲由寬及厚壁星狀菌絲交錯組成；厚壁孢子在孢子果內同步或不同步形成；側枝常形成於孢子的底部，可轉化成孢子或孢間菌絲 (paraphysis-like structure)。本屬形成典型叢枝菌根 (Wu 1993)。

隔膜繡球孢子屬 (*Septoglomus*) (Oehl *et al*. 2011)

本屬產生有顏色的孢子，單獨或疏鬆成堆，產生於土壤中；接著菌絲與孢子的層壁 (laminate layer) 顏色相同，呈管狀，在孢子基部略為窄縮或放寬如漏斗型；隔板 (但有時無) 經常形成近孢子基部處。形成典型叢枝菌根。在台灣常見的 *Glomus constrictum* (圖 12) 與 *Glomus deserticola* 如今已分別更名為 *Septoglomus constrictum* (圖 12) 與 *Septoglomus deserticola*。

進化繡球孢子科 (Claroideoglomeraceae) (Walker & Schüßler 2004)

本科含 1 個屬，(*Claroideoglomus* 進化孢子屬)；形成典型的繡球型孢子；孢壁通常有 3 層；外層會脫離且可與梅澤試劑反應呈粉紅或紫色；中層為半柔軟的層壁 (laminated layer)；內層較薄緊貼著層壁，在接著菌絲內形成隔板；孢子成熟後似「內孢子」；本科的菌種

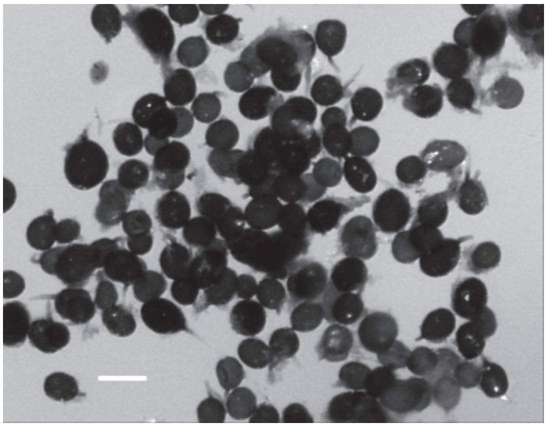

圖 12. 台灣常見的 *Septoglomus constrictum* (= *Glomus constrictum*)。

Fig. 12. *Septoglomus constrictum* (= *Glomus constrictum*), one of the common species in Taiwan (Scale bar = 200 μm).

主要依據孢子大小與中間層壁的顏色來區分；形成典型叢枝菌根；本科的菌種以前皆歸類在繡球孢子屬內，但因本科菌種的 rRNA 小次元體 (SSU) 基因的序列 (CAGYYGGGRAACCRACTAAA; ATTKRCACATCGGTCGTGCCYTAAGGGGYATGAACYRGTGTAGTSA; TAAAAYRGGACGGCATGATTCTATT) 分析結果將其與繡球孢子屬分離並獨立成一進化枝 (clade)。

進化繡球孢子屬 (*Claroideoglomus*) (Walker & Schüßler 2004)

本屬的產孢方式與繡球孢子屬相似，經由菌絲末端膨大後，孢壁再逐漸加厚形成孢子；孢子的外壁常因孢子老化後脫落；在形態上，黏質外壁是孢子最初成形時的第 1 層孢壁，常有有機物質會堆積其上；接著菌絲壁與孢子壁的形成是同步的，孢子壁的形成物質在接著菌絲壁上也可看到；有些菌種其成熟孢子的接著菌絲壁因為太薄了，以致於無法分辨或區隔；本屬特有的 rRNA 小次元體 (SSU) 基因的序列為 CAGYTGGGRAACCRACTAAA; ATTKRCACATCGGTCGTGCC。在台灣常見的 *Glomus claroideum* 與 *Glomus etunicatum* (圖 13) 如今已分別更名為 *Claroideoglomus claroideum* 與 *Claroideoglomus etunicatum* (圖 13)。

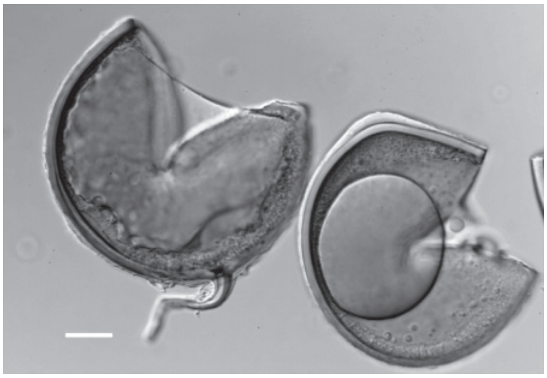

圖 13. 台灣常見菌種 *Claroideoglomus etunicatum* (= *Glomus etunicatum*)。

Fig. 13. *Claroideoglomus etunicatum* (= *Glomus etunicatum*), one of the common species in Taiwan (Scale bar = 20 μm).

多型孢子菌目 (Diversisporales) (Schüßler *et al*. 2001)

本目為地下生真菌；內生菌根具叢枝體，但通常無囊泡；有或無地下生輔助細胞 (auxiliary cell)；於產孢囊泡 (sporiferous saccule) 內產生類似無柄孢子屬的孢子 (acaulosporoid spore) (Morton & Redecker 2001)，或於產孢菌絲 (sporiferous hypha) 的囊狀基座產生類似巨大孢子屬的孢子 (gigasporoid spore)，或於菌絲末端的囊泡壁逐漸加厚後產生似繡球孢子屬的孢子 (glomoid spore)；與其他叢枝菌根菌不同之處在於擁有 (1) rRNA 小次元體 (SSU) 基因的標誌序列 YV<u>R</u>RYW/1-5/NGY<u>Y</u>YGB，本序列與酵母菌的同源位置 658 相當 (*S. cerevisiae* SSU rRNA sequence J01353)。(2) rRNA 小次元體 (SSU) 基因的標誌序列 GTYARDYH<u>M</u>HYY/2-4/GRADRKKYGWCRAC，本序列與酵母菌的同源位置 1346 相當 (*S. cerevisiae* SSU rRNA sequence J01353)。下標線所示核甘酸序列為多型孢子目所特有。

巨大孢子科 (Gigasporaceae) (Morton & Benny 1990)

孢子單獨形成於土壤中或少數種可形成

於根內；孢子自接著菌絲末端膨大的產孢細胞 (sporogenous cell) 發育而成 (圖 14)；所有本科真菌的孢壁 (spore wall)，由 1 個堅硬或半堅硬的層壁 (laminated wall)，1 個永久外壁及 1 個薄內壁組成；孢子可能只具有 1 個孢壁 (*Gigaspora* 屬)；或除孢壁外，還有 1 到 2 個雙層 (bilayered) 的發芽壁 (germinal wall) (圖 15)；孢子發芽時，若無發芽壁，發芽管可以直接自孢壁穿出；或是在發芽壁的最內層所形成的發芽盾，長出發芽管後，穿出孢壁；發芽壁的發育完全獨立，與孢壁的形成無關；輔助細胞 (auxiliary cell) 可自發芽管長出，或是自菌根的根外菌絲長出；輔助細胞會形成疏鬆的聚集；輔助細胞為球形或不規則形，其表面可能平滑或有刺狀突起；輔助細胞在拓殖菌根初期很多，但隨著產孢之後而數量逐漸減少；菌根內菌絲網由管狀菌絲組成，在酸性染劑中著色深；根內菌絲在盆栽植物根內分布廣且多，特別是在宿主植物老化時；菌絲生長不規則，菌絲卷常在感染點 (infection point) 附近形成，但也可能出現在所有拓殖區域 (colonization area)；叢枝體常有較粗的主幹，在透氣良好的土壤中，於草本植物的根內形成 Arum 型的叢枝體多於 Paris 型的叢枝體。根外菌絲透明到暗褐色，常有些區域的菌絲呈不規則或膨大；菌絲壁薄且短暫的菌絲也常出現在土壤或根器官培養中。本科目前有 5 個屬 (*Gigaspora*, *Scutellospora*, *Racocetra*, *Cetraspora*, and *Dentiscutata*) (Oehl *et al*. 2008; Redecker *et al*. 2013)。除 *Gigaspora* 屬之外，其餘的 4 個屬皆具有 1 到 2 個雙層的發芽壁 (圖 15)，本科各屬的定義由於形態特徵與 rDNA 序列差異尚未得到滿意的支撐，雖然仍有些意見分歧 (Morton & Msiska 2010)，但仍維持這 5 個屬的分類 (http://invam.wvu.edu/the-fungi/classification/gigasporaceae)。有關詳細的各屬分類與檢索表可參考 Oehl *et al*. (2008) 的論文。

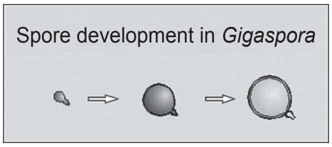

圖 14.　巨大孢子屬的產孢過程。

Fig. 14.　The spore ontogeny of *Gigaspora* (http://invam.wvu.edu/the-fungi/classification/gigasporaceae/gigaspora).

巨大孢子屬 (*Gigaspora*) (Gerdemann & Trappe 1974)

孢子表面平滑沒有突起；孢子含有 1 個雙層孢壁 (spore wall)，內層的孢壁會隨著孢子的成熟而逐漸加厚成層壁 (圖 16)。在孢子發

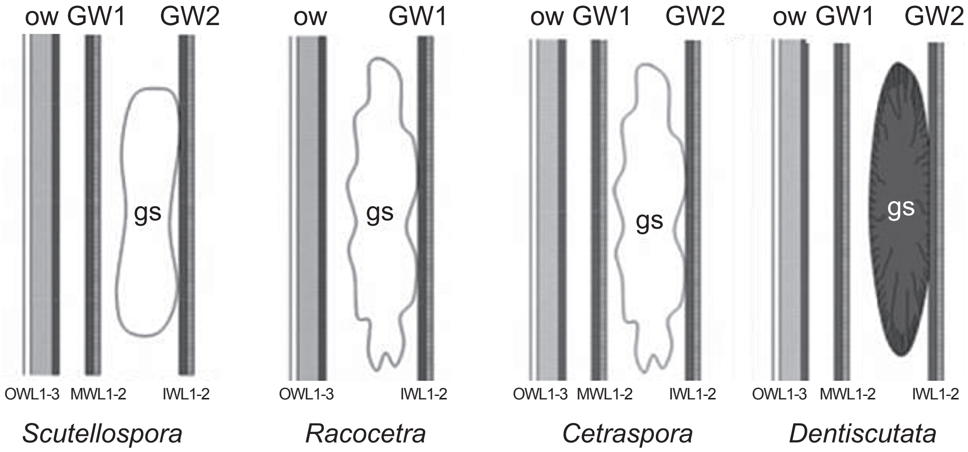

圖 15.　*Scutellospora*、*Racocetra*、*Cetraspora* 和 *Dentiscutata* 屬的孢壁構造比較 (Oehl *et al*. 2008)。

Fig. 15.　Wall structure comparison among the genera of *Scutellospora*, *Racocetra*, *Cetraspora*, and *Dentiscutata* (Oehl *et al*. 2008).

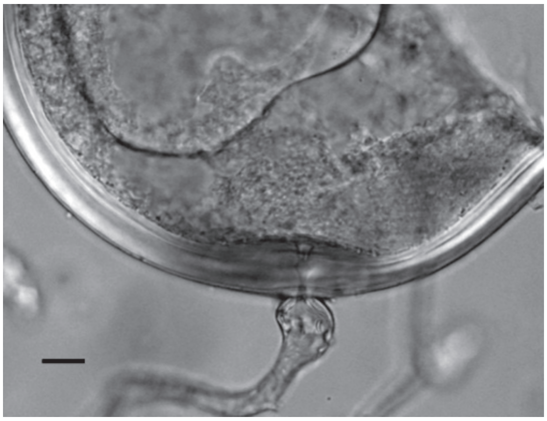

圖 16.　巨大孢子屬 (*Gigaspora margarita*) 的孢壁構造。

Fig. 16.　The spore wall of *Gigaspora margarita* (Scale bar = 20 μm).

芽時，會在層壁靠近細胞質端，再形成另一細薄的內壁，內壁靠細胞質端有瘤狀物出現；發芽管一至多枝，自層壁 (laminated wall) 最內層的疣狀層長出；薄壁的輔助細胞常長在接近根表面的土壤中，其表面具刺狀突起 (圖 17)。

曲折盾孢子屬 (*Racocetra*) (Oehl *et al*. 2008)

本屬孢子從平面上觀其發芽盾呈波浪狀曲折紋路而得名。孢子含有 1 個孢壁及 1 個雙層的發芽壁 (圖 15、圖 18)；孢壁含有 2 個到 3 個堅硬的壁層，其中最外層可能平滑或有紋飾；孢子顏色廣泛，從白色到暗紅黑色；二或更多的發芽管自發芽盾中產生；發芽盾形成於發芽壁之上 (非靠近細胞質的那一面) (圖 18)；輔助細胞自發芽管的分支或根外菌絲長出；輔助細胞的表面凹凸波浪型或鈍狀突起。在台灣常見的 *Scutellospora fulgida* (圖 18)，*Scutellospora gregaria*、*Scutellospora persica* 及 *Scutellospora verrucosa*，如今已分別更名為 *Racocetra fulgida* (圖 18)，*Racocetra gregaria*、*Racocetra persica* 及 *Racocetra verrucosa*。

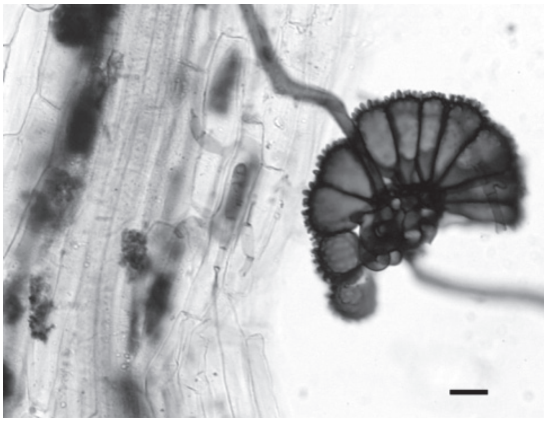

圖 17.　巨大孢子屬的輔助細胞具有刺狀突起。

Fig. 17.　The auxiliary cells of *Gigaspora*. Notes its surface with narrow projections (Scale bar = 20 μm).

淡盾孢子屬 (*Cetraspora*) (Oehl *et al*. 2008)

孢子表面平滑或具有紋飾；孢子具有 1 個雙層的孢壁 (SW) 及 2 個雙層的發芽壁 (GW) (圖 15、圖 19)；發芽管形成於 1 個永固的扁形發芽盾，發芽盾總是與最內層的發芽壁相連；發芽盾無色，或淡黃色；薄壁的輔助細胞，平滑或具有波浪狀突起；輔助細胞常形成於接近根表面的菌絲。

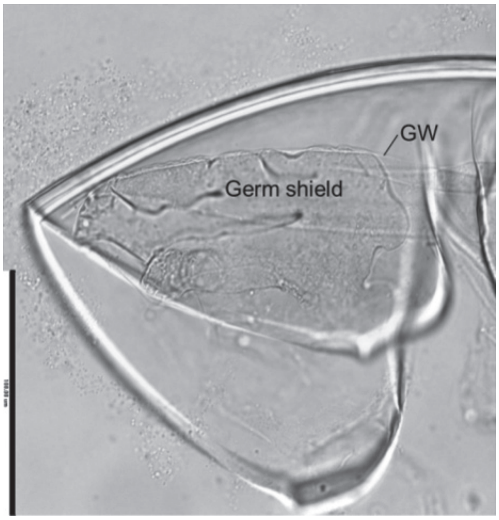

圖 18.　*Racocetra fulgida* 的發芽盾 (germ shield) 形成於孢壁與內發芽壁 (GW) 之間。

Fig. 18.　Germ shield of *Racocetra fulgida*, formed between spore wall and a germinal wall (GW) (Scale bar = 100 μm).

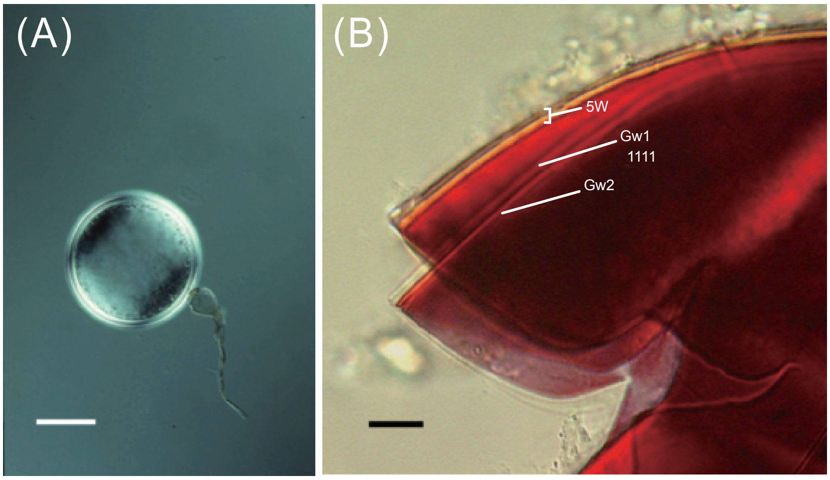

圖 19.　(A) 台灣常見的 *Cetraspora pellucida*。(B) 孢壁內層可被梅澤試劑染成紅紫色，第 2 個發芽壁 (Gw2) 也可被梅澤試劑染成紅紫色。

Fig. 19.　(A) The spore of *Cetraspora pellucid* (Scale bar = 100 μm). (B) Note the inner layer of spore wall (SW) stained reddish purple in Melzer's reagent, and the inner germinal wall (GW2) stained reddish purple in Melzer's reagent too (Scale bar = 30 μm).

裂緣盾蓋孢子屬 (*Dentiscutata*) (Oehl *et al*. 2011)

孢子表面平滑或具有紋飾；孢子具有 1 個雙層的孢壁 (SW) 及 2 個雙層的發芽壁 (GW) (圖 15、圖 20)；發芽管形成於 1 個永固的扁形發芽盾，發芽盾總是與最內層的發芽壁相連；發芽盾的外緣常有鋸齒狀的裂紋；薄壁的輔助細胞，平滑或具有波浪狀突起；輔助細胞常形成於接近根表面的菌絲。形成典型的叢枝菌根。本屬在台灣常見的菌種有 *Dentiscutata heterogama* (圖 20) 及 *Dentiscutata nigra*。這些菌種常見於海邊的植物根圈土壤中。

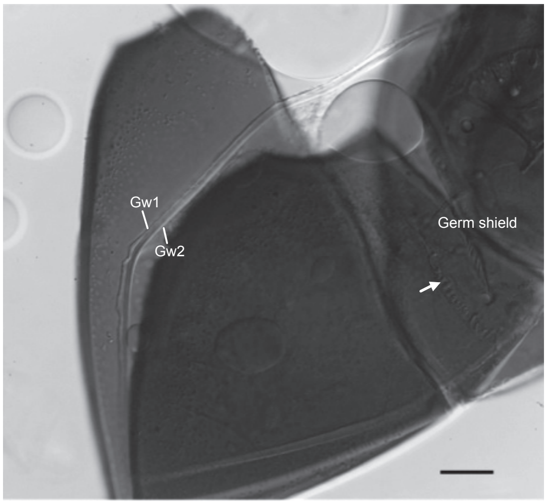

圖 20.　台灣海邊植物根圈土壤中常見的 *Dentiscutata heterogama*。箭頭所示為裂紋狀發芽盾 (Germ shield)。Gw1、Gw2 為兩個雙層的發芽壁。

Fig. 20.　One of the common species in the rhizosphere of beach plants, *Dentiscutata heterogama*. Note the dentate germ shield (arrow) and two bi-layered germinal walls (Gw1, Gw2) (Scale bar = 10 μm).

盾蓋孢子屬 (*Scutellospora*) (Walker & Sanders 1986)

孢子具有 1 個孢壁及 2 個雙層發芽壁；孢壁由 2 個到 3 個永久層組成，孢壁的外層和 (或) 層壁可能平滑或具有突起等紋飾；孢子的顏色範圍廣，從白色到暗紅黑色；具有 1 到多枝來自發芽盾的發芽管；發芽盾形成於最接近孢子細胞質的內層發芽壁的表面；在可形成發芽盾的發芽壁與孢壁之間仍存在 1 個到 2 個柔軟的發芽壁。輔助細胞來自於孢子的發芽管和根外菌絲，會糾結成堆；輔助細胞的表面幾乎平滑，或有微波浪狀的突起。台灣常見的菌種有 *Scutellospora calospora*。

無柄孢子科 (Acaulosporaceae) (Morton & Benny 1990)

本科的孢子均產生在產孢囊泡 (sporiferous saccule) 的柄 (接著菌絲) 內或柄上，其孢子形成的過程均是一致的 (圖 21)；一開始先形成產孢囊泡，最後產生完整分化的孢子；孢子具有 1 個雙層的孢壁，1 個透明雙層的外柔軟壁 (發芽壁 1) 及 1 個透明雙層的柔軟內壁 (發芽壁 2)；孢子發育完成後，發芽包 (germination orb) 總在這內壁 (發芽壁 2) 上形成；孢子壁的外層是來自於產孢囊泡的壁，該壁隨著時間會逐漸分解脫落，所以最終孢子形成之後，沒有任何接著菌絲；整個孢子是無柄的；孢子的內含物與產孢囊泡柄分隔是由於孢壁的第 2 層 (層壁) 向內堆積後形成；產孢囊泡柄與孢子接觸的區域，因為留有一道圓形或卵圓形脊狀痕跡，稱之為產孢疤痕 (cicatrix)；孢壁的第 2 層是層壁，該壁因菌種的不同而有不同的顏色與紋飾；有些種可能有第 3 層緊貼著層壁，可以利用梅澤試劑來進一步區分出來；發芽管是自圓形發芽包長出，源自於孢子內的發芽壁，透明且常被孢子內的油滴所掩蓋，但在老孢子則因內含物清澈而容易被觀察到。

無柄孢子屬 (*Acaulospora*) (Gerdemann & Trappe 1974)

本屬早期定義為可將孢子產生在產孢囊泡柄的側面的菌種，但現在分類已將內柄生孢子屬 (*Entrophospora*) 與無柄生孢子屬 (*Acaulospora*) 合併，因為內柄生孢子 ‘entrophosporoid spore’ 被視為趨同演化的性狀。在過去 *Entrophospora*、*Acaulospora* 兩屬的差異僅在於孢子在產孢囊泡上的位置不同而已。事實上，在產孢囊泡柄內產孢的特性在演化上出現多次，並不能定義為單源演化的結果，這種產孢特性應是一種趨同演化的產物。因為在 SSU 及 LSU 的 rDNA 序列分析指出 *Entrophospora kentinensis* (圖 22)、*Entrophospora Columbiana* 等與其他的 *Acaulospora* 屬類似；另 *Entrophospora schenckii* 則與 *Archaeospora* 屬近似。在台灣酸性森林土壤中常見的 *Entrophospora infrequens* (圖 23)，目前因 rDNA 序列的分析與其他的 *Entrophospora* 與 *Acaulospora* 屬不同，暫時保留在 *Entrophospora* 屬中。

和平孢子科 (Pacisporaceae) (Walker *et al*. 2004)

本科唯一的屬，和平孢子屬 (*Pacispora*) 在 2004 年由 Oehl 和 Sieverding 兩人所命名。Walker & Schüßler (2004) 稍後也發表了類似的分系觀念，將本屬歸類於 Gerdemanniaceae 科和 *Gerdemannia* 屬 (Walker *et al*. 2004)。但根據命名法這一新的科名與屬名均屬無效的命名。可是當時 Oehl & Sieverding (2004) 並未

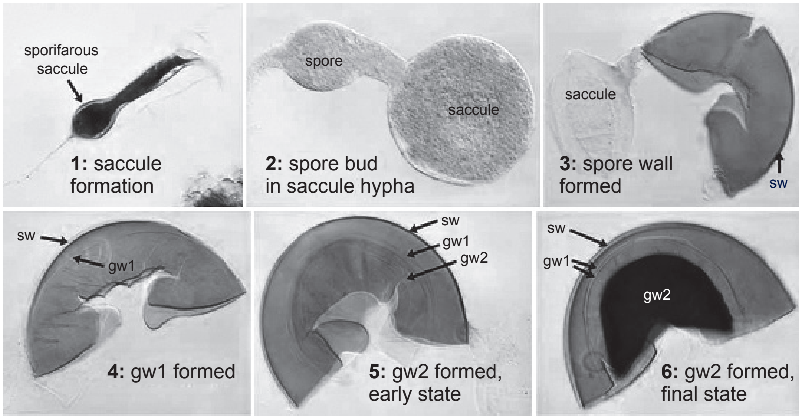

圖 21. *Acaulospora colombiana* 產孢與各層壁的發育過程。

Fig. 21. The spore ontogeny and wall development of *Acaulospora colombiana* (http://invam.wvu.edu/the-fungi/classification/acaulosporaceae).

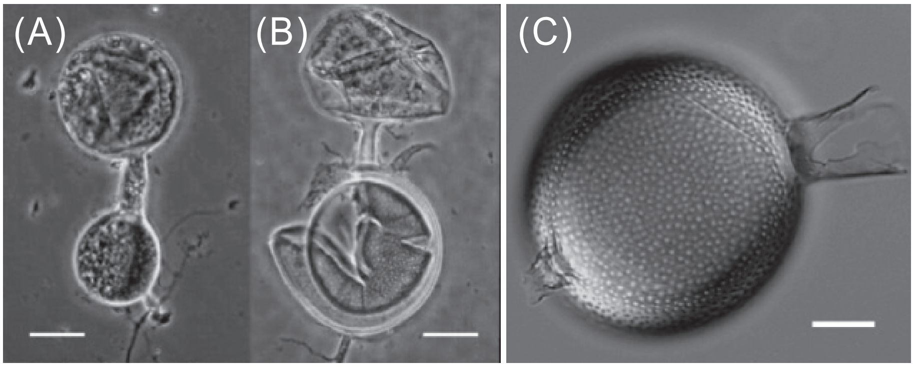

圖 22. 在台灣所發現的 *Entrophospora kentinensis*，如今已更名為 *Acaulospora kentinensis*。(A) 產孢囊泡的細胞質向下移動，菌絲柄膨大。(B) 成熟的孢子 (已壓破) 形成於產孢囊泡柄內。(C) 成熟未壓破的孢子，尚可見到左右各一個產孢疤痕。

Fig. 22. *Entrophospora kentinensis*, originally described from Taiwan, now renamed as *Acaulospora kentinensis*. (A) The cytoplasm contents of sporiferous saccule was empty, and the saccule stalk getting swollen (Scale bar = 50 μm). (B) A mature spore (broken one) formed within the stalk of sporifeous saccule (Scale bar = 50 μm). (C) A mature spore showing two cicatrices (Scale bar = 25 μm).

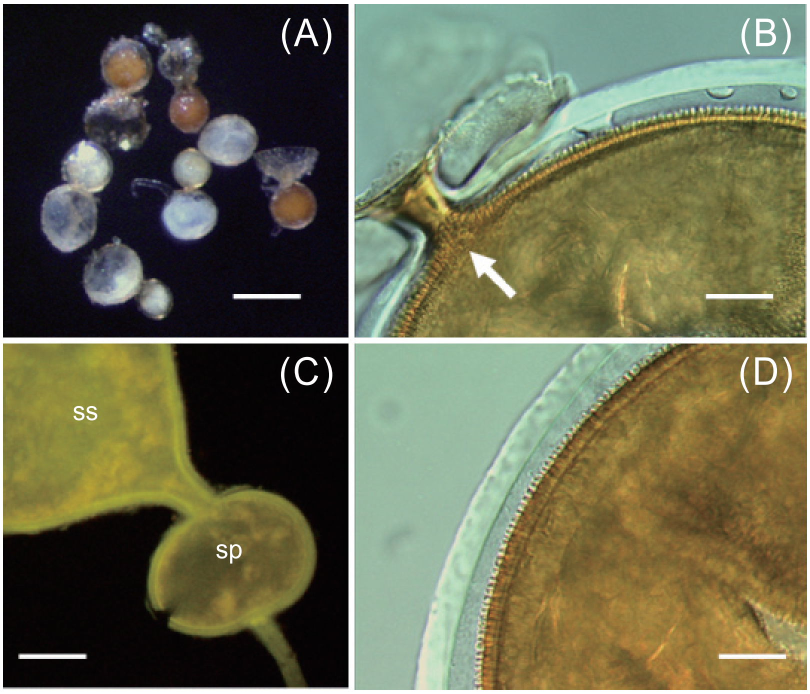

圖 23. 台灣酸性森林土壤中常見的 *Entrophospora infrequens*. (A) 產孢囊泡 (白色) 與孢子 (褐色)。(B) 孢子壁褐色物質仍部分停留在產孢囊泡的頸部，形成「瓶塞」 (arrow)。(C) 在紫外線螢光顯微鏡下觀察可見產孢囊泡 (SS) 壁與孢子 (SP) 壁 (下部圓形) 相連。孢子形成於囊泡柄內。(D) 產孢囊泡壁透明無色，孢子壁褐色表面具鈍刺狀突起。

Fig. 23. *Entrophospora infrequens*, one of the common species in Taiwan forest soil. (A) Sporiferous saccule (white) and spore (brown) (Scale bar = 250 μm). (B) Brown wall material accumulated in the neck of sporiferous saccule (SS) forming a ‘plug’ (arrow) (Scale bar = 20 μm). (C) Under ultraviolet fluorescent microscope, showing that spore (SP) was formed inside the neck of SS. Outer spore wall interconnected with SS wall (Scale bar = 50 μm). (D) Wall of SS, hyaline, and spore wall brown, with projections (Scale bar = 20 μm).

主張新的科名來安置和平孢子屬，於是 Walker & Schüßler 在同一年，根據合法命名的和平孢子屬 (*Pacispora*) 的特徵，再提出和平孢子科 (Pacisporaceae) 的命名。本科產生似繡球孢子 (glomoid spores)，但孢子有 2 個壁群，其中至少有 1 個彈性的發芽壁；發芽時在發芽壁上形成發芽包 (圖 24)。

和平孢子屬 (*Pacispora*) (Oehl & Sieverding 2004)

本屬不產生孢子果；孢子具有獨特的型態特徵；孢子具有 1 個多層的孢壁以及 1 個厚而半柔軟的內發芽壁；本發芽壁上可形成發芽包；形成典型的囊叢枝菌根 (圖 24、圖 25)。本屬目前有 6 個種，原先是歸類在繡球孢子屬 (*Glomus*)，因為這些菌種均有繡球孢子屬的特徵；可是除此之外，這 6 個種卻擁有半柔軟的發芽壁以及與無柄孢子科 (Acaulosporaceae) 和巨大孢子科 (Gigasporaceae) (除 *Gigaspora* 屬外) 相同的發芽方式 (Oehl & Sieverding 2004)。

多型孢子菌科 (Diversisporaceae) (Walker & Schüßler 2004)

孢子類似繡球孢子；有些種的孢子會被長自接著菌絲 (subtending hypha) 的包被包裹， 包被內可能含 2–3 個孢子；本科的命名主要依據其特有的 rRNA 小次元體的基因序列 GGCTCATTYGRRTYTS、ACYCATTRY-CAGGCTTAAT 及 TTGGCATTTAGYCA， 這些序列分別與酵母菌 (*Saccharomyces cerevisiae* SSU rRNA sequence J01353) 的同源位置 487、648、1389 相當。

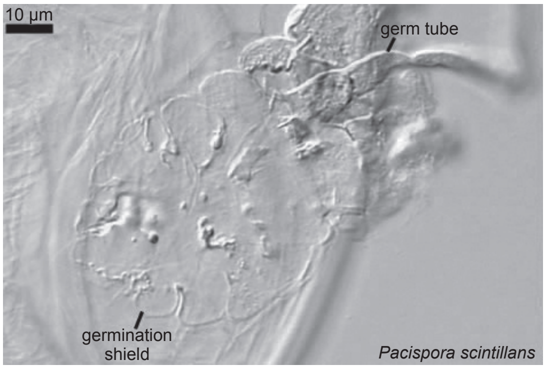

圖 24. *Pacispora scintillans* 的發芽盾 (germination shield) 與發芽管 (germ tube)。

Fig. 24. Germination shield and germ tube of *Pacispora scintillans* (http://www.zor.zut.edu.pl/Glomeromycota/pages/Pac_-scintillans-124-9-PV-_jpg.htm).

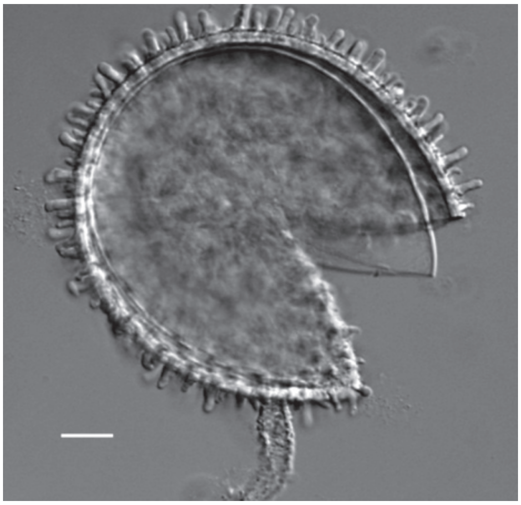

圖 25. 在台灣所發現的 *Glomus chimonobambusae* 如今已更名為 *Pacispora chimonobambusae*。

Fig. 25. *Glomus chimonobambusae*, originally described from Taiwan and found in bamboo forest, is now renamed as *Pacispora chimonobambusae* (Scale bar = 30 μm).

多型孢子屬 (*Diversispora*) (Walker & Schüßler 2004)

本屬的孢子繡球型，其孢壁皆具有非堅硬的層壁；這些層壁在壓力下會約略膨脹而改變厚度；本屬擁有 rRNA 小次元體 (SSU) 基因的標誌序列 CYCATTRGYCAGGCTTAATT-GTC、TATTGGCATTTAGYCA 及 CTTTGGAT-TRGGGTTTAGGGRTC，這些序列與酵母菌 (*Saccharomyces cerevisiae* SSU rRNA sequence J01353) 的同源位置 6459、1387、1673 相當。在台灣所發現的 *Glomus tortuosum* 如今已更名為 *Diversispora tortuosa* (圖 26)。

瑞德克氏菌屬 (*Redeckera*) (Walker & Schüßler 2004)

產孢方式與繡球菌屬及其他產生類繡球孢

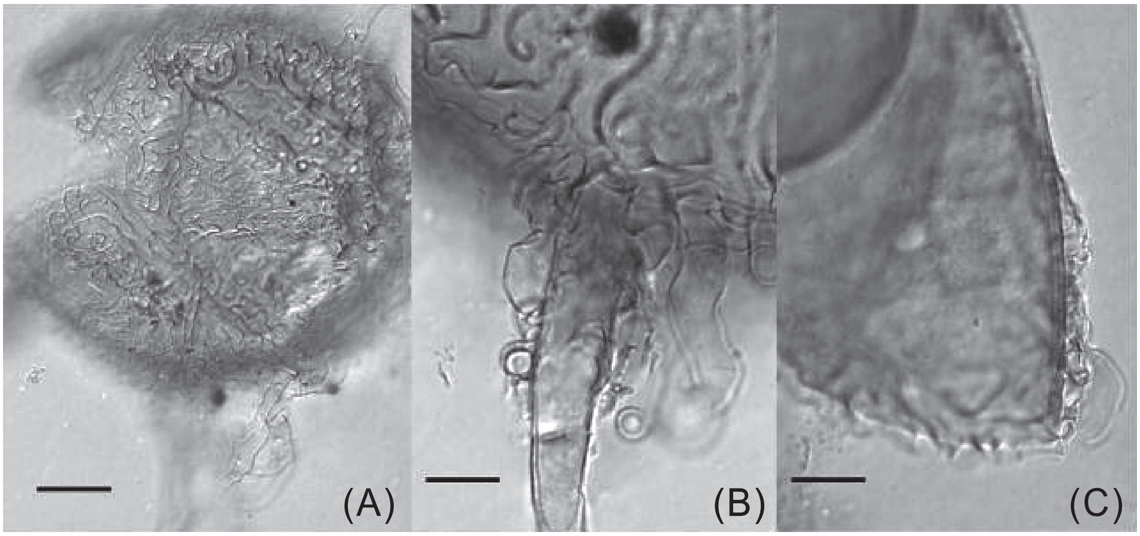

圖 26. 在台灣所發現的 *Glomus tortuosum* 如今已更名為 *Diversispora tortuosa*。(A) 孢子被菌絲胞被包裹。(B) 菌絲胞被係由孢子接著菌絲壁長出後向孢子延伸所形成。(C) 孢壁外有菌絲包裹。

Fig. 26. *Glomus tortuosum*, recovered from the rhizosphere of peanut in Taiwan, is now renamed as *Diversispora tortuosa*. (A) Spore surrounded by peridial hyphae (Scale bar = 30 μm). (B) Peridial hyphae branching from attached hypha and growing toward spore surface (Scale bar = 10 μm). (C) Spore wall covered by peridial hyphae (Scale bar = 10 μm).

子的分類單元類似；截至目前為止，所有本屬的菌種皆產生相當大的孢子果且有包被包裹；常出現在囓齒類動物的腸道內；本屬菌種均擁有 rRNA 小次元體 (SSU) 的標誌序列 ARKTYTGGKMGCGGYAACGTRA。在台灣鼠類腸道內常發現的 *Glomus fulvum* 與 *Glomus pulvinatum* 如今已分別更名為 *Redeckera fulvum* 與 *Redeckera pulvinatum* (圖 27、圖 28)。

古生孢子菌目 (Archaeosporales) (Schüßler *et al*. 2001)

本目為地下生真菌；可與光自營原核生物形成胞內 (endocytosymbioses) 共生或形成具有叢枝體的菌根；有或無囊泡，孢子透明無色或不與梅澤試劑 (Melzer's reagent) 產生反應；似繡球孢子屬的孢子 (glomoid spore) 單獨或疏鬆成團地產生於土壤中或土壤表層；似無柄孢子屬的孢子 (acaulosporoid spore) 單獨形成於土壤中，有無產生密集的孢子堆則尚未可知；與其他叢枝菌根菌不同之處在於擁有 rRNA 小次元體 (SSU) 基因的標誌序列 YCTATCYKYCTGGTGAKRCG，本序列與酵母菌的同源位置 691 相當 (*S. cerevisiae* SSU rRNA sequence J01353)。下標線所示核甘酸序列為古生孢子目所特有。

圖 27. 在台灣鼠類腸道內常發現的 Glomus fulvum 如今已分別更名為 Redeckera fulvum。

Fig. 27. Glomus fulvum, one of the common species found inside the intestine tract of mouse in Taiwan, is now renamed as Redeckera fulvum (Scale bar = 20 μm).

土管菌科 (Geosiphonaceae) (Engler & Gilg 1924)

這真菌只分布在德國思沛莎山區及奧地利

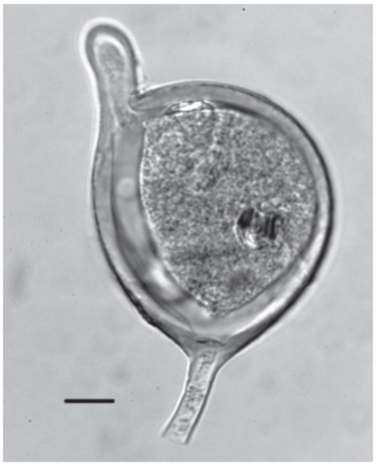

圖 28. 在台灣鼠類腸道內常發現的 *Glomus pulvinatum*，如今已分別更名為 *Redeckera pulvinatum*。

Fig. 28. *Glomus pulvinatum*, one of the common species found inside the intestine tract of mouse in Taiwan, is now renamed as *Redeckera pulvinatum* (Scale bar = 20 μm).

等地，極為稀少；與之共生的藍綠藻 (*Nostoc*) 長在土管菌 (*Geosiphon pyriformis*) 的管袋內；這種共生真菌與共生植物間的大小與位置，與一般的叢枝菌根相反；本科真菌並沒有跡象顯示會與其他高等植物共生，所以本項共生組合與其他繡球菌門的各科間在生態上有明顯的區隔。

土管菌屬 (*Geosiphon*) (Wettstein 1915)

土管菌屬是土管菌科唯一的屬；該屬含單一種 *Geosiphon pyriformis*，1849 首次由 Kützing 命名，當時的種名為 *Botrydium pyriforme*。Wettstein (1915) 已發現本菌含有幾丁質，而且幾丁質為真菌細胞壁的重要成分。但他認為本生物為多細胞核藻類，且細胞內有藍綠藻共生，所以重新定名為 *Geosiphon pyriforme*。Knapp (1933) 首次認為本生物應屬於真菌並將之描述為地衣，有藍綠藻與之共生。本屬為繡球菌門唯一不與陸地植物形成共生叢枝菌根的成員。*Geosiphon pyriformis* 是念珠藻 (*Nostoc*) 的共生體。*Geosiphon-Nostoc* 的共生並不符合地衣的定義，因為它們是細胞內的結合。就功能與演化上的意義來看，*Geosiphon-Nostoc* 的共生更接近於叢枝菌根的共生，而非地衣藻類與真菌間的共生。*Geosiphon-Nostoc* 的共生是目前所知唯一真菌與藍綠藻內共生的例子。*Geosiphon* 的膨大菌絲，單細胞，多核，如管狀的囊泡，0.5–2 mm，長在土壤的表面。真菌管狀囊泡，約 2/3 的地上部內有藍綠藻的絲狀細胞與異形細胞 (heterocyst)，其餘的 1/3 充滿油脂顆粒。*Geosiphon pyriformis* 的孢子約 250 μm，形成於菌絲的末端或菌絲中段，與繡球菌門的其他成員一樣。*Geosiphon* 細胞內有一特化的袋狀共生體構造 (symbiosome) 可供藍綠藻，特別是念珠藻 (*Nostoc punctiforme*) 棲息之用。*Geosiphon* 的袋狀共生體光合作用旺盛，同時也具有固定大氣中的氮的作用。共生體是由真菌的細胞膜為捕捉藍綠藻而向細胞質內摺後形成，內含藍綠藻。共生體內的藍綠藻較一般外面環境中自由生活的營養細胞大 6–8 倍。針對藻菌間介面的厚度，幾丁質含量，以及壁的超微結構等而言，真菌 (*Geosiphon pyriformis*) 與念珠藻 (*Nostoc*) 之間的共生介面就如同高等植物與真菌的菌根介面一般。但 *Geosiphon pyriformis* 並不會形成叢枝菌根。

雙型孢子科 (Ambisporaceae) (Walker *et al*. 2007)

本科叢枝菌根菌可同時產生類繡球孢子與類無柄孢子；這兩種不同型的孢子未必同時產生於盆栽培養之中；類繡球孢子，透明，具有雙層孢壁，單獨產孢或形成孢子堆；類無柄孢子可從產孢囊泡 (sporiferous saccule) 的頸部向外延伸所形成的短柄 (pedicel) 中形成；所有類無柄孢子具有 1 個多層的孢壁與 1 個半柔軟的內壁；孢壁外層，有龜裂表面，迷宮型的皺褶，或兩者兼有，但厚度不穩定或容易分解；孢壁的中層與內層是本科各菌種的鑑定依據；本科與古生孢子菌目 (Archaeosporales) 的其他科之間的差異在於產生雙型孢子，特殊的孢子形態及 rDNA 序列；菌根主要有叢枝體，少有囊泡產生，菌根染色很淡，不易觀察。

雙型孢子屬 (*Ambispora*) (Walker *et al*. 2007)

類無柄孢子型：孢子源自於產孢囊泡頸部

的側生短柄 (pedicel)；產孢囊泡是由生殖菌絲的末端膨大後形成；菌絲膨大的現象似乎在孢子開始形成的時候停止。孢子脫離產孢囊泡後，可能還會保留原短柄；但假如短柄斷裂處是在與孢子底部接著部位的話，那孢子會看起來是無柄的；孢子有 1 個 3 層的孢壁；外層會有不同程度的分解或完全脫落；中間層與產孢囊泡頸部的短柄內層壁相連；內層則具有特殊的性質；中層壁與內層壁的性狀對於本屬的種的鑑定非常關鍵；孢子不具有薄而彈性「發芽壁」。

類繡球孢子型：本型孢子為典型的繡球狀，大小範圍廣 (即使在單一族群內)；顏色從透明到乳白色；具有 1 個雙層的孢壁，孢壁與接著菌絲的雙層壁相連續；類繡球孢子的形態特徵在各種之間不具有鑑別價值。在台灣酸性土壤中，特別是在陽明山國家公園常見的 *Glomus leptotichum*，如今更名為 *Ambispora leptotica* (圖 29、圖 30)。

古生孢子菌科 (Archaeosporaceae) (Morton & Redecker 2001)

在本科的兩個屬，其與其他繡球孢子科

圖 29. 在台灣酸性土壤中，特別是在陽明山國家公園常見的 *Glomus leptotichum* 如今更名為 *Ambispora leptotica*。類無柄孢子 (acaulosporoid spore) 可被梅澤試劑染成紅褐色。

Fig. 29. *Glomus leptotichum*, one of the common species in acidic soil, especially in National Yangmingsan Park, is renamed as *Ambispora leptotica*. Note the acaulosporoid spore is stained reddish brown in Melzer's reagent (Scale bar = 20 μm).

圖 30. *Ambispora leptotica* 透明無色的類無柄孢子。

Fig. 30. Hyaline, acaulosporoid spore of *Ambispora leptotica* (Scale bar = 50 μm).

相關的科 (含類繡球菌科) 的菌種，主要區別在於 rRNA 小次元體基因的標誌序列 TCTCKKYTTCGGYSGAGTCC；本序列與酵母菌 (*Saccharomyces cerevisiae* SSU rRNA sequence J01353) 的同源位置 227 相當。

古生孢子屬 (*Archaeospora*) (Morton & Redecker 2001)

本屬皆產生孢子於產孢囊泡 (sporiferous saccule) 的接著菌絲 (subtending hypha) 的側面或接著菌絲內；雖然產孢模式與無柄孢子科的菌種相似 (趨同演化的性狀)，但孢子內部構造卻非常獨特。孢子不具有彈性的內壁。本屬目前有兩個種，因其形態特徵曾分別被命名為 *Acaulospora trappei* 和 *Entrophospora schenckii*。但在當時的報告即已指出這 2 種並不具有 *Acaulospora* 和 *Entrophospora* 屬專有的「雙層發芽壁」的特徵。根據 rDNA 的小次元體 (SSU) 的序列數據顯示 *Ar. trappei* 應較為原始 (Redecker *et al*. 2000)。Sieverding & Oehl (2006) 根據孢子產生在產孢囊泡的接著菌絲柄內，而將 *E. schenckii* 歸類在 *Intraspora* 屬，但由於產孢位置的特徵是一趨同演化的結果，因此無法支持其歸類在新屬的主張。Schüßler & Walker (2010) 根據其 rDNA 的小次元體序列與 *Archaeospora* 的相關性， 將本菌種更名為 *Ar. shenckii*。

類繡球菌目 (Paraglomerales) (Schüßler *et al*. 2001)

本目為地下生真菌；所形成的菌根含有叢枝體，根內菌絲體 (intraradical mycelium)，但少有囊泡；可產生透明無色的繡球狀孢子；與其他叢枝菌根菌不同之處在於擁有 rRNA 小次元體 (SSU) 基因的標誌序列 GCGAAGCGT-CATGGCCTTAACCGGCCGT，本序列與酵母菌的同源位置 703 相當 (*S. cerevisiae* SSU rRNA sequence J01353)。下標線所示核甘酸序列為類繡球菌目所特有；本標誌序列係來自目前該目兩個種的數據，若將來發現更多本目的菌種，本序列有可能再次修正。

類繡球菌科 (Paraglomaceae) (Morton & Redecker 2001)

分子特徵是用來鑑定本科真菌的主要依據：(1) 擁有特殊的單株抗體 B5，F8；(2) 5.8 S 核醣體 3' 端的標誌序列 GGCATGTCT-GTTTGAGGGCACCA，將本科與其他繡球菌科的菌種區隔；(3) 引子序列 TGCTA-AATAGCCAGGCTGY (ARCH 1311) 可選擇性地將本科真菌的核醣體 DNA 進行增幅。本科菌種所形成的菌根中，其叢枝體無法被酸性染劑染色，或是不易著色；叢枝體的主幹小於 44 µm 寬，在末端有細的分支；囊泡不易觀察到；根內菌絲染色較叢枝體淺；細胞內菌絲有些呈緊密纏繞 3–10 µm 寬；菌絲若沒有捲曲纏繞的部分，有不規則分叉 2–8 µm 寬；根外菌絲則染色深 2–6 µm 寬，常沿根表面大量生長並且產孢。本科菌種大多產生單一孢子，很少有 2–3 個孢子聚集成堆的；孢壁構造與繡球菌屬相同，孢壁的層次構造延伸到接著菌絲；孢子與接著菌絲隔絕，主要是因孢壁的最內層加厚所致；本科孢子發芽並未觀察到，但認為應與其他繡球菌科的所有菌種一樣，發芽管是自接著菌絲的內腔長出，很少是穿過孢壁的。

類繡球菌屬 (*Paraglomus*) (Morton & Redecker 2001)

目前本屬只有 2 個種，之前因孢子的型態皆歸類在繡球菌屬 (*Glomus*)；本屬菌根構造不易染色等形態特徵是鑑定本屬的重要線索。在台灣溫室中常見的汙染菌種 *Glomus occultum* 如今更名為 *Paraglomus occultum* (圖 31)。

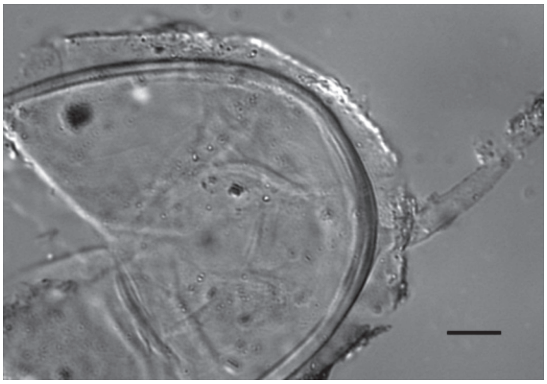

圖 31. 在溫室培養菌根菌的過程中常見的污染菌種 *Glomus occultum*，如今更名為 *Paraglomus occultum*。

Fig. 31. *Glomus occultum*, one of the common species, causing contamination in greenhouse cultures, is renamed as *Paraglomus occultum* (Scale bar = 10 µm).

引用文獻

Berkeley, M. J. and C. E. Broome. 1873. Enumeration of the fungi of Ceylon. Part II. J. Linn. Soc. London Bot. 14:137.

Cavalier-Smith, T. 1998. A revised six-kingdom system of life. Biol. Rev. 73:203–266.

Engler, A. and E. Gilg. 1924. Syllabus der Pflanzenfamilien. Verlag von Gebrüder Borntraeger. Berlin. 420 pp.

Gerdemann, J. W. and J. M. Trappe. 1974. The *Endogonaceae* in the Pacific Northwest. Mycol. Mem. 5:1–76.

Hildebrandt, U., K. Janetta, and H. Bothe. 2002. Towards growth of arbuscular mycorrhizal fungi independent of a plant host. Appl. Environ. Microbiol. 68:1919–1924.

Knapp, E. 1933. Über *Geosiphon pyriforme* Fr. Wettst., eine intrazelluläre Pilz-Algen-Symbiose. Ber. Dtsch. Bot. Ges. 51:210–217.

Morton, J. B. and D. Redecker. 2001. Two families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. Mycologia 93:181–195.

Morton, J. B. and G. L. Benny. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): A new order, Glomales, two new suborders, Glo-

mineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. Mycotaxon 37:471–491.

Morton, J. B. and Z. Msiska. 2010. Phylogenies from genetic and morphological characters do not support a revision of Gigasporaceae (Glomeromycota) into four families and five genera. Mycorrhiza 20:483–496.

Nicolson, T. H. and N. C. Schenck. 1979. Endogonaceous mycorrhizal endophytes in Florida. Mycologia 71:178–198.

Oehl, F. and E. Sieverding. 2004. *Pacispora*, a new vesicular arbuscular mycorrhizal fungal genus in the Glomeromycetes. J. Appl. Bot. 78:72–82.

Oehl, F., F. A. de Souza, and E. Sieverding. 2008. Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming Glomeromycetes. Mycotaxon 106:311–360.

Oehl, F., G. A. da Silva, B. T. Goto, and E. Sieverding. 2011. *Glomeromycota*: Three new genera and glomoid species reorganized. Mycotaxon 116:75–120.

Pirozynski, K. A. and Y. Dalpé. 1989. Geological history of the Glomaceae, with particular reference to mycorrhizal symbiosis. Symbiosis Rehovot 7:1–35.

Redecker, D., A. Schüßler, H. Stockinger, S. L. Stürmer, J. B. Morton, and C. Walker. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23:515–531.

Redecker, D., J. B. Morton, and T. D. Bruns. 2000. Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). Mol. Phylogenet. Evol. 14:297–301.

Schenck, N. C. and G. S. Smith. 1982. Additional new and unreported species of mycorrhizal fungi (*Endogonaceae*) from Florida. Mycologia 74:77–92.

Schenck, N. C., J. L. Spain, E. Sieverding, and R. H. Howeler. 1984. Several new and unreported vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Colombia. Mycologia 76:685–699.

Schüßler, A. 2002. Molecular phylogeny, taxonomy, and evolution of *Geosiphon pyriformis* and arbuscular mycorrhizal fungi. Plant Soil 244:75–83.

Schüßler, A. and C. Walker. 2010. The *Glomeromycota*. A species list with new families and new genera. http://www.arbuscular-mycorrhiza.net/Schuessler&Walker2010_Glomeromycota.pdf (visit on 6/30/2015)

Schüßler, A., D. Schwarzott, and C. Walker. 2001. A new fungal phylum, the *Glomeromycota*: Phylogeny and evolution. Mycol. Res. 105:1413–1421.

Tulasne, L. R. and C. Tulasne. 1845. Fungi nonnulli hypogaei, novi. v. minus cogniti act. G. Bot. Ital. 2:35–63.

Walker, C. and A. Schüßler. 2004. Nomenclatural clarifications and new taxa in the Glomeromycota. Mycol. Res. 108:981–982.

Walker, C. and F. E. Sanders. 1986. Taxonomic concepts in the Endogonaceae: III. The separation of *Scutellospora* gen. nov. from *Gigaspora* Gerd. & Trappe. Mycotaxon 27:169–182.

Walker, C., J. Blaszkowski, D. Schwarzott, and A. Schüßler. 2004. *Gerdemannia* gen. nov., a genus separated from *Glomus*, and Gerdemanniaceae fam. nov., a new family in the Glomeromycota. Mycol. Res. 108:707–718.

Walker, C., M. Vestberg, F. Demircik, H. Stockinger, M. Saito, H. Sawaki, I. Nishmura, and A. Schüßler. 2007. Molecular phylogeny and new taxa in the *Archaeosporales* (*Glomeromycota*): *Ambispora fennica* gen. sp. nov., *Ambisporaceae* fam. nov., and emendation of *Archaeosporaceae*. Mycol. Res. 111:137–153.

Wettstein, F. V. 1915. *Geosiphon* Fr. Wettst, eine neue, interessante Siphonee. Österr. Bot. Z. 65:145–156.

Wu, C. G. 1993. Glomales of Taiwan: IV. A monograph of *Sclerocystis* (Glomaceae). Mycotaxon 49:327–349.

Wu, C. G., Y. S. Liu, Y. L. Hwuang, Y. P. Wang, and C. C. Chao. 1995. *Glomales* of Taiwan V. *Glomus chimonobambusae* and *Entrophospora kentinensis*, spp. nov. Mycotaxon 53:283–294.

Wu, C. G. and Z. C. Chen. 1986. The Endogonaceae of Taiwan I. A preliminary investigation on Endogonaceae of bamboo vegetation at Chi-tou areas, central Taiwan. Taiwania 31:65–87.

附錄 繡球菌門雙叉檢索表

Appendix A dichotomous key for Glomeromycota

1. 菌根染色不易或顏色淡；孢子透明或顏色淡 2

1’. 菌根染色深；孢子顏色多樣 3

2. 孢子似繡球狀，無色；擁有核醣體小次元體 (SSU) 基因的標誌序列 GCGAAGCGTCATG-GCCTTAACCGGCCGT，本序列與酵母菌 (*S. cerevisiae* SSU rRNA sequence J01353) 的同源位置 703 相當 .... Paraglmerales (Paraglomeraceae, *Paraglomus*)

2’. 可與光自營原核生物形成胞內共生 (endocytosymbioses) 或形成具有叢枝體的菌根；孢子形狀多樣，或似繡球狀，或似無柄孢子；擁有核醣體小次元體 (SSU) 基因的標誌序列 YCTAT CYKYCTGGTGAKRCG，本序列與酵母菌 (*S. cerevisiae* SSU rRNA sequence J01353) 的同源位置 691 相當 .... Archaeosporales (4)

3. 孢子繡球狀，單一或孢子果；與其他叢枝菌根真菌的區隔，主要在於其核醣體的小次元體 (SSU) 基因的標誌序列 YTRRY/2-5/RYYARGTYGNCARCTTCTTAGAGGGACTATCGGTG-TYTAACCGRTGG，本序列與酵母菌 (*S. cerevisiae* SSU rRNA sequence J01353) 的同源位置 1353 相當 .... Glomerales (6)

3’. 孢子繡球狀，無柄孢子狀，或巨大孢子狀；有或無土生輔助細胞；擁有核醣體小次元體 (SSU) 基因的標誌序列 YVRRYW/1-5/NGYYYGB 及 GTYARDYHMHYY/2-4/GRADRKKYGWC-RAC，本序列分別與酵母菌 (*S. cerevisiae* SSU rRNA sequence J01353) 的同源位置 658 及 1346 相當 .... Diversisporales (11)

4. 形成叢枝菌根 5

4’. 不形成叢枝菌根，與藍綠藻產生胞內共生 .... Geosiphonaceae (*Geosiphone*)

5. 可產生類繡球孢子與類無柄孢子；但類無柄孢子內不具有彈性發芽壁 .... Ambisporaceae (*Ambispora*)

5’. 產生孢子於產孢囊泡的接著菌絲的側面或接著菌絲柄內；主要區別在於核醣體的小次元體 (SSU) 基因之標誌序列 TCTCKKYTTCGGYSGAGTCC，本序列與酵母菌 (*S. cerevisiae* SSU rRNA sequence J01353) 的同源位置 227 相當 .... Archaeosporaceae (*Archaeospora*)

6. 孢子繡球型，產生於地表或接近地表的土壤中；孢子單獨、孢子堆、或產生於具有包被或沒有包被的孢子果中；其核醣體小次元體 (SSU) 基因的標誌序列為 TGTYADGGCAYYRCA-CYGG .... Glomeraceae (7)

6’. 形成典型的繡球型孢子；孢壁通常有 3 層；外層會脫離可與梅澤試劑反應呈粉紅或紫色；中層為層壁；內層較薄，在接著菌絲內形成隔板；孢子成熟後似「內孢子」；具有特殊核醣體小次元體 (SSU) 基因的標誌序列 (CAGYYGGGRAACCRACTAAA；ATTKRCACATCG-GTCGTGCCYTAAGGGGYATGAACYRGTGTAGTSA；TAAAAYRGGACGGCATGATTC-TATT) .... Claroideoglomeraceae (*Claroideoglomus*)

7. 孢子繡球狀；其核醣體小次元體 (SSU) 基因的標誌序列為 GGTACGYACTGGTATCATTGG 和 TCGGCTGTAAAAGGCYYTTG .... *Glomus*

7'. 孢子繡球狀，但不符合 7 的描述 8

8. 孢子非透明無色，單獨產生於土壤中或孢子聚集成堆 (1- 約 20 個孢子)，四周有粗菌絲網包裹；孢子基部如漏斗狀；孢壁 2–3 層，外壁透明且隨著孢子的成熟而逐漸脫離；孢子通常在孢子基部的下緣有一隔板；孢子形成的方式與繡球孢子屬同；其核醣體小次元體 (SSU) 基因的標誌序列為 CGGTCATGCCGTTGGTATGY .... *Funneliformis*

8'. 孢子產孢於根內；或有色孢子的層壁與接著菌絲顏色相同；或形成球型、卵形、半圓形孢子果等 9

9. 繡球狀孢子單獨產生於土壤中，或成團聚集於土壤或根內 .... *Rhizophagus*

9'. 有色孢子的層壁與接著菌絲顏色相同；或形成球型、卵形、半圓形孢子果等 10

10. 本屬皆產生孢子果；球形、亞球形或半球形；孢子果具包被或裸露；孢子果具一或多枝菌絲柄；厚壁孢子自孢子果中心叢向外形成；孢子與孢子緊緊相鄰成 1 層；中心叢菌絲由寬而厚壁星狀菌絲交錯組成；厚壁孢子在孢子果內同步或不同步形成；側枝常形成於孢子的底部，可轉化成孢子或孢間菌絲 .... *Sclerocystis*

10'. 有色孢子的接著菌絲與孢子的層壁 (laminate layer) 顏色相同，管狀；在孢子基部略為窄縮或放寬如漏斗型；隔板 (但有時無) 經常形成近孢子基部處 .... *Septoglomus*

11. 孢子單獨形成於土壤中或少數種可形成於根內；孢子自接著菌絲末端的產孢細胞發育而成；所有本科真菌的孢壁由 1 個堅硬或半堅硬的層壁，1 個永久外壁及 1 個薄內壁組成； 孢子可能具有 1 個孢壁；或除孢壁外，還有 1–3 個雙層的發芽壁；孢子發芽時，若無發芽壁，發芽管可以直接自孢壁上的疣穿出；或是自發芽壁的最內層所形成的發芽盾長出發芽管後，穿出孢壁；發芽壁的發育完全獨立與孢壁無關；輔助細胞可自發芽管長出或是自菌根的根外菌絲長出；輔助細胞會形成疏鬆的聚集；輔助細胞球形或不規則形，其表面可能平滑也可能刺狀突起 .... Gigasporaceae (14)

11'. 孢子不是自產孢細胞發育而成；孢子產生在產孢囊泡的柄 (接著菌絲) 內或柄上；或產生類繡球孢子並具有柔軟內發芽壁；或類繡球孢子會被長自接著菌絲的包被包裹 12

12. 孢子產生在產孢囊泡的柄 (接著菌絲) 內或柄上；一開始先形成產孢囊泡，最後產生完整分化的孢子；孢子具有 1 個雙層的孢壁，1 個透明雙層的外柔軟壁 (發芽壁 1) 及 1 個透明雙層的柔軟內壁 (發芽壁 2)；孢子發育完成後，發芽包總在這內壁上形成；孢子壁的外層是來自於產孢囊泡的壁，該壁隨著時間會逐漸分解脫落，所以最終孢子形成之後，沒有任何接著菌絲，整個孢子是無柄的 .... Acaulosporaceae (*Acaulospora*)

12'. 產生類繡球孢子並具有柔軟內發芽壁；或類繡球孢子會被長自接著菌絲的包被包裹 13

13. 產生類繡球孢子，除孢壁外，還有具彈性的內發芽壁；發芽時，會在發芽壁上形成發芽包並產生發芽管 .... Pacisporaceae (*Pacispora*)

13'. 產生類繡球孢子，有些種的孢子會被長自接著菌絲的包被包裹；本科的命名主要依據其特有的核醣體小次元體 (SSU) 基因的標誌序列 GGCTCATTYGRRTYTS、 ACYCATTRYCAG-GCTTAAT 和 TTGGCATTTAGYCA，這些序列分別與酵母菌 (*S. cerevisiae* SSU rRNA sequence J01353) 的同源位置 487、648、1389 相當 .... Diversisporaceae (18)

14. 孢子表面平滑沒有突起；孢子含有 1 個雙層孢壁；發芽管 1 至多枝，自層壁最內層的疣狀層長出；薄壁的輔助細胞常長在接近根表面的土壤中，其表面具刺狀突起 .... *Gigaspora*

14’. 孢子發芽時會形成發芽盾 15

15. 孢子除孢壁外，還有 2 個以上的雙層發芽壁 16

15’. 孢子除孢壁外，只有 1 個雙層的發芽壁 .... *Racocetra*

16. 發芽盾顏色深且外緣有齒狀裂紋者 .... *Denticutata*

16’. 發芽盾無色或顏色較淺 17

17. 發芽盾構造簡單，1 到 2 個裂瓣 (lob) .... *Scutellospora*

17’. 發芽盾構造複雜，具有多個裂瓣，每個裂瓣又具有 4 個或更多的區隔，因此可形成多條發芽管 .... *Cetraspora*

18. 孢子繡球型，其孢壁皆具有非堅硬的層壁；這些層壁在壓力下會約略膨脹而改變厚度；本屬擁有核醣體小次元體 (SSU) 基因的標誌序列 CYCATTRGYCAGGCTTAATTGTC、TATTGGCATTTAGYCA 和 CTTTGGATTRGGGTTTAGGGRTC................................ *Diversispora*

18’. 孢子繡球型，產生大型孢子果且有包被包裹；本屬菌種均擁有核醣體小次元體 (SSU) 基因的標誌序列 ARKTYTGGKMGCGGYAACGTRA .... *Redeckera*

A New Definition for Glomeromycota

Chi-Guang Wu[1] and Su-Chen Lin[2,*]

Abstract

Wu, C. G. and S. C. Lin. 2016. A new definition for Glomeromycota. J. Taiwan Agric. Res. 65(3):238–260.

In this paper, new definitions for Glomeromycota and its related orders, families, and genera were introduced. Meanwhile, species names for arbuscular mycorrhizal fungi mass produced by industries or used in field tests were reclassified based on these new definitions. A new dichotomous key for Glomeromycota was provided.

Key words: Arbuscular mycorrhizal fungi, Glomeromycota, Dichotomous key.

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Research Article

Effect of Neutralized Phosphorus Acid on Powdery Mildew and Fruit Quality of Muskmelon (*Cucumis melo* L.) in Greenhouse

Chen-Yu Lin[1], Kan-Shu Chen[2], Ya-Suie Hwang[3], Hui-Ling Lo[1], and Cheng-Fang Hong[4,*]

Abstract

Lin, C. Y., K. S. Chen, Y. S. Hwang , H. L. Lo, and C. F. Hong. 2016. Effect of neutralized phosphorus acid on powdery mildew and fruit quality of muskmelon (*Cucumis melo* L.) in greenhouse. J. Taiwan Agric. Res. 65(3):261–268.

Greenhouse-grown muskmelon (*Cucumis melo* L.) is one of the most high value fruit crops in Taiwan. Powdery mildew, caused by *Podosphaera xanthii*, can infect leaves and vines of muskmelon and result in huge loss of quality and yield. In this study, the efficiency of two concentrations (1 and 2 g L^{-1}) of neutralized phosphorus acid (NPA) applied at two spraying schedules (7- and 14-day intervals) on controlling powdery mildew were compared with triflumizole and a water control in greenhouse. Disease severity of powdery mildew among different treatments were evaluated every 7-d after fruit set. The fresh weight of fruit, flesh thickness and total soluble solid content were evaluated as fruit quality index after harvest. When NPA was applied at a 7-d interval, the efficiency on controlling powdery mildew were not significantly different ($P = 0.05$) with triflumizole. In contrast, when NPA was applied at 1 g L^{-1} at 14-d interval, the disease severity was not significantly different with the non-treatment control. Overall, the 7-d spraying schedule has a better control on powdery mildew no matter what concentration of NPA was applied. In the fruit quality analysis, NPA sprayed at 2 g L^{-1} at 14 days and 1 g L^{-1} at 7 days significantly increased the fresh weight of fruits when compared with untreated control. However, there was no significant differences ($P = 0.05$) between treatments on the flesh thickness and total soluble solid content of muskmelon except for applying NPA at 2 g L^{-1}/14-d in the second trial.

Key words: *Cucumis melo* L., Muskmelon, Neutralized phosphorus acid (NPA), Powdery mildew, Non-fungicide control.

INTRODUCTION

Greenhouse-grown muskmelon (*Cucumis melo* L.) is a high value crop in Taiwan. Powdery mildew, caused by *Podosphaera xanthii* (Castag.) Braun & Shishkoff, is a major disease which can infect leaves, vines and decrease the fruit quality and yield in muskmelon production (McCreight *et al*. 1987). In most cucurbit crops, application of fungicides is the major practice for managing powdery mildew (McGrath 2007). In recent years, many

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studies are seeking alternative methods for managing powdery mildew due to increasing concerns of environmental sustainability, food safety, and necessity to reduce the application of fungicides (McGrath 2001; Mkhize *et al*. 2013). Several alternative methods have been reported effectively control of the powdery mildew on cucurbit in greenhouse and application of phosphorus salts is one of the methods (Keinath & DuBose 2012). Phosphorus acid has been found to induce resistance of crops and has been used for protecting plants against several plant pathogens (Guest 1984; Panicker & Gangadharan 1999).

Phosphites is an alkali metal salt of phosphorous acid which is also a source of plant phosphorus nutrition or an agricultural fungicide (McDonald *et al*. 2001). Recent research showed that phosphites or phosphorus acid confer induced resistance in plants and provide systemic protection against many plant pathogens (Choudhary *et al*. 2007). Some studies showed that phosphites confer resistance against *Phytophthora infestans* and *Fusarium solani* in potato (Lobato *et al*. 2008). Other reports also demonstrated the efficiency on controlling powdery mildew by foliar sprays of phosphate salts on different crops, such as cucumber (Reuveni *et al*. 1996), nectarine, mango, and grapevines (Reuveni & Reuveni 1995).

Defense mechanisms of crops conferred by phosphites includes direct and indirect pathways. High level concentration of phosphate at the infection site could directly reduce fungal growth (Smillie *et al*. 1989). Indirect pathway is the stimulation of systemically acquired resistance and prevention of infection by fungal pathogens (Percival *et al*. 2009; Bock *et al*. 2012). Furthermore, several studies indicated the production and accumulation of phenolic compounds and phytoalexin which can reduce disease severity (Saindrenan *et al*. 1988; Danie & Guest 2006). The control of Phytophthora disease in avocado and pineapple, potato tuber rots, and maize downy mildew were demonstrated related with the accumulation of phenolic compounds after application of phosphorus acid (Pegg *et al*. 1990; Panicker & Gangadharan 1999; Johnoson *et al*. 2004). Application of phosphites can also increase pectin content in periderm and cortex tissue in potato tubers (Olivieri *et al*. 2012). On cauliflower, potassium phosphonate can activate β-1,3-glucanase and induced localized resistance to downy mildew (Bécot *et al*. 2000).

However, concentrations and spraying schedules of phosphites may affect their efficiency against diseases as well as the growth of crops (Bock *et al*. 2012). Previous study showed that application of phosphites at least three times could offer better protection against apple and pear scab (Percival *et al*. 2009). Also, different concentrations of phosphites showed different growth inhibition on potato pathogens (Lobato *et al*. 2010). In conventional practice, muskmelon is cultivated on the ground in fields (Ibarra *et al*. 2001). In order to produce high quality muskmelon, greenhouse production technology has been widely applied (Rodriguez *et al*. 2007; Cantliffe *et al*. 2009). In Taiwan, vertical cultivation of muskmelon in greenhouse creates a high planting density and low ventilation environment in which muskmelon is very susceptible to powdery mildew (Wang *et al*. 2004). Application of phosphorus acid maybe an alternative option to reduce the loss caused by powdery mildew in greenhouse-grown muskmelon and to reduce the application of fungicides. However, information on effective concentrations of phosphorus acid and spraying schedules is still limited. Also, most studies only focused on evaluating the efficiency of disease control but ignored the effect of phosphorus acid on fruit quality. The objectives of this study are: (1) to evaluate the efficiency of different treatments of neutralized phosphorus acid (NPA) on controlling powdery mildew, and (2) to investigate the fruit quality of muskmelon after application of NPA under vertical cultivation model in the greenhouse.

MATERIALS AND METHODS

Variety and cultivation of muskmelon

Two trials were conducted in the greenhouse at Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute, Kaohsiung City, during the 2009 and 2010 fall growing seasons, respectively. Muskmelon (*Cucumis melo* L.) cv. 'Honey world' seeds used in this study were obtained from Know-You Seed Co. Ltd., Taiwan. Seeds were sown in 50-well pots (4.5 cm diameter per well) containing peat moss for 25 d. Then, seedlings were transplanted to cultivation containers (8 m in length, 30 cm in width and 30 cm in depth). In each container, peat moss and coconut fiber were mixed (1 : 1; v/v) as cultural substrate. Two containers were laid side by side and set up 30 cm above ground. Plant density was 7.4 plants m^{-2} with 45 cm between plants and 30 cm between rows. Vertical trellis was used for vine growing. Top leaves and shoots of the main vine were trimmed when the plant has more than 30 internodes. Only 28 leaves were retained during fruit development and secondary vines grew between 13th to 16th nodes of the main vine was kept for the fruit set. After successful fruit set, only one fruit per plant was developed and secondary vines without fruits were trimmed off. To fertilize the plants, Yamazaki nutrient solution was applied in the dripping irrigation system during the growth period of both trials (Yamazaki 1982). In the 2012 trial, 200 g of biotech organic fertilizer No.1 (Taiwan fertilizer Co. Ltd., Taiwan) was applied before planting and blooming. Total cultivation period was 85 d since seeds were sown and the fruit development period was 42 d after hand pollination.

Effect of neutralized phosphorus acid on powdery mildew

The effect of neutralized phosphorus acid (NPA) on disease severity of powdery mildew was assessed 13 d after hand pollination in each year. Phosphorus acid was neutralized with potassium hydroxide by dissolving equal weight of both chemicals in water (1 : 1; w/w) (Ann 2001). Six treatments were compared in this study, including (1) tap water as non-treatment control, (2) NPA at 2 g L^{-1} at 7-d interval, (3) NPA at 2 g L^{-1} at 14-d interval, (4) NPA at 1 g L^{-1} at 7-d interval, (5) NPA at 1 g L^{-1} at 14-d interval, and (6) 30% triflumizole WP (Soda Co. Ltd., Japan) 3,000× at 7-d interval. Trials were randomized completely block designed (RCBD) with four plants in each replicate and three replicates in each treatment. For the evaluation of disease severity, eight leaves from the lower node parts (no. 5th to 8th) and middle node parts (no. 13th to 16th) of the main vine were investigated. The disease severity was scored using a 0-4 scale, where 0 means healthy leaves; 1 = diseased area is less than 25% of the leaf; 2 = diseased area is between 26–50% of the leaf; 3 = diseased area is between 51–75% of the leaf; and 4 = diseased area is higher than 75% of the leaf. The disease severity was calculated using the following equation:

$$\text{Disease severity (\%)} = [\Sigma(N_{0\text{-}4} \times n)/(N \times 4)] \times 100$$

Where $N_{0\text{-}4}$ represents the disease severity of each leaf; n = the number of leaves in each disease severity; and N = the number of total leaves assessed. Each plant was assessed at a 7-d interval. For 7-d interval treatments, NPA and triflumizole were applied at 14th, 21th, 28th, and 35th d after hand pollination. For the 14-d interval treatments, NPA was applied on 14th and 28th day after hand pollination.

Evaluation of fruit quality

After the last assessment of disease severity, fruits were harvested and the fruit weight (g), flesh thickness (cm) and total soluble solid (°Brix) were used to evaluate the fruit quality. For ripening after harvest, the harvested fruit was stored at room temperature for 4 d and to-

tal soluble solid was measured.

Statistical analysis

The disease severity and fruit quality data were subjected to analysis of variance using the SAS-EG (SAS Enterprise Guide 4.1 system). Treatment means separation was performed using the least significant difference (LSD) at $P = 0.05$.

RESULTS AND DISCUSSION

The first assessment of disease severity of powdery mildew was carried out 13 days after hand pollination in both trials. The disease severity was not significantly different among all treatments in the first and the second assessments of both trials. At the 27th day, application of NPA at 1 g L^{-1} at 7-d interval and triflumizole significantly ($P = 0.05$) reduced the disease severity of powdery mildew when compared with the non-treatment control in both trials. As the disease pressure increased between 34th to 41th day, only NPA applied at 7-d interval consistently and significantly reduced the disease severity of powdery mildew in both trials. Also, the efficiency on controlling powdery mildew on muskmelon was not significantly different with triflumizole when NPA was applied at both concentrations at 7-d interval (Fig. 1 and 2). However, in the 14-d interval treatments, only NPA applied at 2 g L^{-1} significantly controlled the expansion of powdery mildew in the second trial. This result revealed that the application schedule is more crucial than the concentration when applying NPA for the control of powdery mildew (Fig. 3).

Effect of NPA on the fruit quality of muskmelon was evaluated. In the 2012 trial, the fruit weight in all treatments were higher than that in 2009 trial due to an extra application of biotech organic fertilizer. Besides, the fruit weight is significantly higher ($P = 0.05$) than water control when NPA was applied at 2 g L^{-1} at 14-d interval or at 1 g L^{-1} at 7-d interval in both trials (Table 1). The NPA treatments were obviously promoted fruit development except for NPA treated at 1 g L^{-1} at 14-d interval in which the fruit weight was not significantly different with that of the non-treated control. Compared with the triflumizole, the

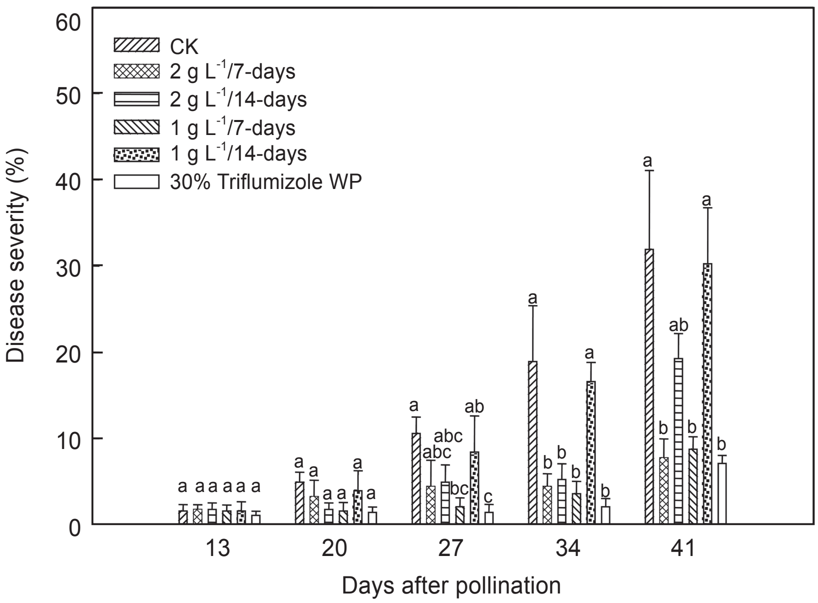

Fig. 1. Effect of different NPA treatments on powdery mildew of muskmelon after pollination in 2009.

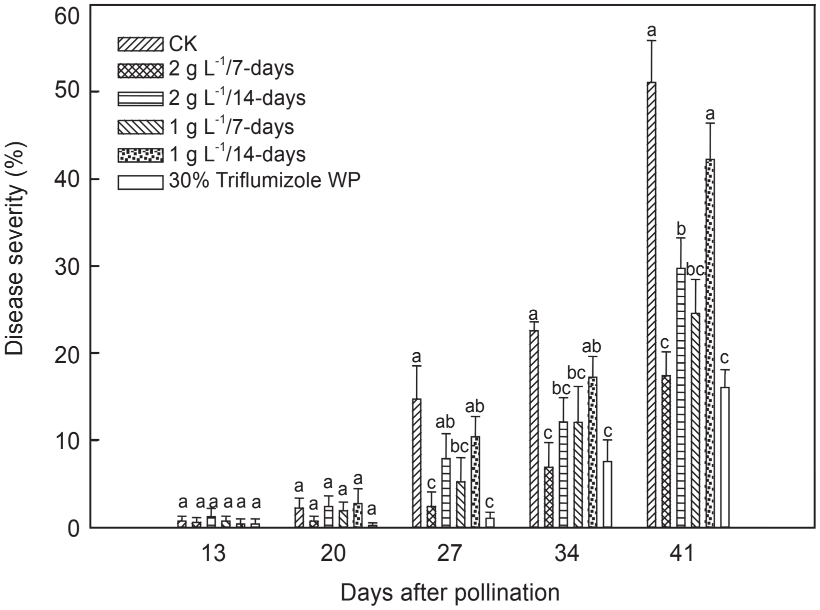

Fig. 2. Effect of different NPA treatments on powdery mildew of muskmelon after pollination in 2012.

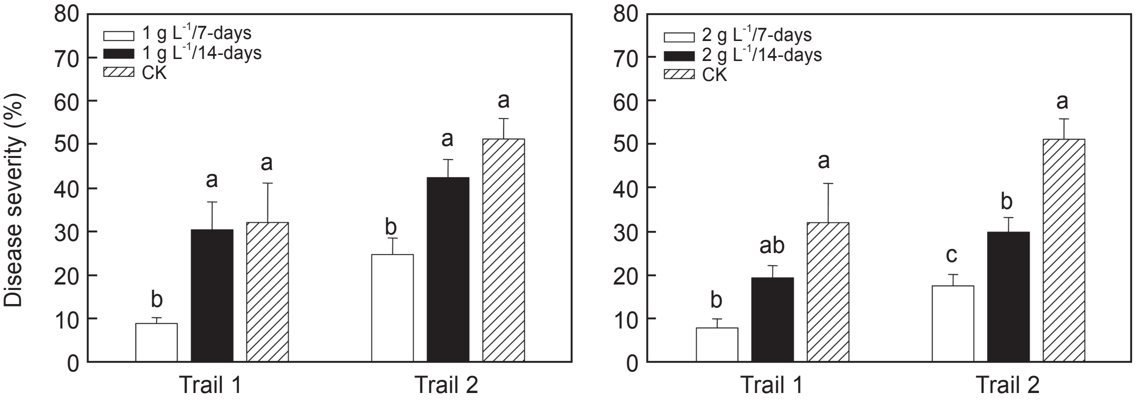

Fig. 3. Effect of different NPA spraying schedules on powdery mildew in both trials.

fresh weight of muskmelon was significantly higher when NPA was applied at 2 g L^{-1} at 14-d interval in both trials. For the flesh thickness and total soluble solids, there were not significant differences among all treatments in the first trial. However, application of NPA at 2 g L^{-1} at 14-d interval significantly increased the flesh thickness of muskmelon in the second trial.

The fruit weight of muskmelon increases rapidly after pollination and increases slightly before harvest (Lingle & Dunlap 1987; Miccolis & Saltveit 1991). Therefore, effective control of powdery mildew during early to middle period of fruit development after pollination is important. Previous studies indicated that defense mechanisms of crops conferred by phosphites include direct and indirect pathways (Smillie *et al.* 1989; Percival *et al.* 2009; Bock *et al.* 2012). Induction of defense mechanism could result in production of pathogenesis-related (PR) proteins and accumulation of phe-

Table 1. Effect of various NPA treatments on fruit quality of greenhouse-grown muskmelon.

Treatment	Trial 1			Trial 2		
	Fruit fresh weight (g)	Fruit flesh thickness (cm)	Total Soluble Solid (°Brix)	Fruit fresh weight (g)	Fruit flesh thickness (cm)	Total Soluble Solids (°Brix)
CK	1183.8 c[z]	3.2 a	12.0 a	1323.3 d	3.5 b	11.0 a
2 g L^{-1}/7-day	1263.2 bc	3.2 a	12.0 a	1476.7 ab	3.8 b	12.1 a
2 g L^{-1}/14-day	1439.1 a	3.4 a	12.3 a	1581.7 a	4.1 a	11.5 a
1 g L^{-1}/7-day	1346.4 ab	3.1 a	12.3 a	1535.0 ab	3.8 b	12.2 a
1 g L^{-1}/14-day	1235.0 bc	3.2 a	12.0 a	1346.7 cd	3.8 b	12.1 a
30% Triflumizole WP	1259.4 bc	3.1 a	12.6 a	1445.0 bc	3.7 b	11.0 a

[z] Means in each column followed by different letters are significantly different according to Fisher's LSD test at 5%.

nolic compounds or phytoalexins which may not persist for a long time (Choudhary *et al.* 2007; Mkhize *et al.* 2013). In this study, NPA applied at 7-d interval significantly reduced the disease severity of powdery mildew on muskmelon no matter what concentration was applied. However, the efficiency on controlling powdery mildew was significantly lower when NPA was applied at 14-d interval than that applied at 7-d interval. This result indicates a short term defense mechanism is involved in against powdery mildew after application of NPA.

Bock *et al.* (2012) reported applying phosphites at 2-week interval slightly increase the fruit weight in some pecan cultivars. In this study, NPA treatments could significantly increase fruit weight except for those treated with 1 g L^{-1} at 14-d interval in which the fruit weight was not significantly different with the non-treated control. The fresh weight of muskmelon was significantly higher when NPA was applied at 2 g L^{-1} at 14-d interval than that of triflumizole. For the flesh thickness and total soluble solids, no significant differences were found among all treatments in the first trial. However, application of NPA at 2 g L^{-1} at 14-d interval significantly increased the flesh thickness of muskmelon in the second trial. Apparently, application of NPA did not consistently affect the flesh thickness and total soluble solids of muskmelon in this study. Li *et al.* (2012) reported that the amount of irrigation water could significantly affect the flesh thickness and total soluble solids in greenhouse conditions. In these two trials, all treatments used the same dripping irrigation schedule and showed no significant differences among treatments except for 2 g L^{-1} at 14-d interval of NPA.

This study demonstrated that application of NPA significantly reduced the disease severity of powdery mildew on muskmelon in greenhouse and showed NPA as a potential alternative for chemical fungicide. Based on the results, weekly application of NPA at 1 g L^{-1} has more advantages on the fruit quality and offers a promising control of powdery mildew on muskmelon in greenhouse.

REFERENCES

Ann, P. J. 2001. Control of plant diseases with non-pesticide compound-phosphorous acid. Plant Prot. Bull. 10:147–154. (in Chinese with English abstract)

Bécot, S., E. Pajot, D. L. Corre, C. Monot, and D. Silué. 2000. Phytogard® (K_2HPO_3) induces localized resistance in cauliflower to downy mildew of crucifers. Crop Prot. 19:417–425.

Bock, C. H., T. B. Brenneman, M. W. Hotchkiss, and B. W. Wood. 2012. Evaluation of a phosphite fungicide to control pecan scab in the southeastern USA. Crop Prot. 36:58–64.

Cantliffe, D. J., J. M. Harty, N. L. Shaw, S. A. Sargent, and P. J. Stoffella. 2009. Greenhouse production of 'charentais' -type cantaloupes (*Cucumis melo* L. var. *cantalupensis*). Proc. Fla. State Hort. Soc. 122:281–285.

Choudhary, D. K., A. Prakash, and B. N. Johri. 2007. Induced systemic resistance (ISR) in plants: Mecha-

nism of action. Indian J. Microbiol. 47:289–297.

Danie, R. and D. Guest. 2006. Defense responses induced by potassium phosphonate in *Phytophthora palmivora*-challenged *Arabidopsis thaliana*. Physiol. Mol. Plant Pathol. 67:194–201.

Guest, D. I. 1984. Modification of defense responses in tobacco and capsicum following treatment with Fosetyl-Al [Aluminium tris (o-ethyl phosphonate)]. Physiol. Plant Pathol. 25:125–134.

Ibarra, L., J. Flores, and J. C. Díaz-Pérez. 2001. Growth and Yield of muskmelon in response to plastic mulch and row covers. Sci. Hort. 87:139–145.

Johnoson, D. A., D. A. Inglis, and J. S. Miller. 2004. Control of potato tuber rots caused by oomycetes with foliar applications of phosphorous acid. Plant Dis. 88:1153–1159.

Keinath, A. P. and V. B. DuBose. 2012. Controlling powdery mildew on cucurbit rootstock seedling in the greenhouse with fungicides and biofungicides. Crop Prot. 42:338–344.

Li, Y. J., B. Z. Yuan, Z. L. Bie, and Y. Kang. 2012. Effect of drip irrigation criteria on yield and quality of muskmelon grown in greenhouse conditions. Agric. Water Manage. 109:30–35.

Lingle, S. E. and J. R. Dunlap. 1987. Sucrose metabolism in netted muskmelon fruit during development. Plant Physiol. 84:386–389.

Lobato, M. C., F. P. Olivieri, E. A. González Altamiranda, E. A. Wolski, G. R. Daleo, D. O. Caldiz, and A. B. Andreu. 2008. Phosphite compounds reduce disease severity in potato seed tubers and foliage. Eur. J. Plant Pathol. 122:349–358.

Lobato, M. C., F. P. Olivieri, G. R. Daleo, and A. B. Andreu. 2010. Antimicrobial activity of phosphites against different potato pathogens. J. Plant Dis. Prot. 117:102–109.

McCreight, J. D., M. Pitrat, C. E. Thomas, A. N. Kishaba, and G. W. Bohn. 1987. Powdery mildew resistance genes in muskmelon. J. Amer. Soc. Hort. Sci. 112:156–160.

McDonald, A. E., B. R. Grant, and W. C. Plaxton. 2001. Phosphite (phosphorous acid): Its relevance in the environment and agriculture and influence on plant phosphate starvation response. J. Plant Nutr. 24:1505–1519.

McGrath, M. T. 2001. Fungicide resistance in cucurbit powdery mildew: Experience and challenges. Plant Dis. 85:236–245.

McGrath, M. T. 2007. Managing cucurbit powdery mildew and fungicide resistance. Acta Hort. 731:211–216.

Mkhize, N., J. P. Bower, I. Bertiling, and N. Mathaba. 2013. Response of citrus physiology to phosphorus acid and silicon as elicitors of induced disease resistance. Acta Hort. 1007:135–141.

Miccolis, V. and M. E. Saltveit. 1991. Morphological and physiological changes during fruit growth and maturation of seven melon cultivars. J. Amer. Soc. Hort. Sci. 166:1025–1029.

Olivieri, F. P., M. L. Feldman, M. F. Machinandiarena, M. C. Lobato, D. O. Caldiz, G. R. Daleo, and A. B. Andreu. 2012. Phosphite applications induce molecular modifications in potato tuber periderm and cortex that enhance resistance to pathogens. Crop Prot. 32:1–6.

Panicker, S. and K. Gangadharan. 1999. Controlling downy mildew of maize caused by *Peronosclerospora sorghi* by foliar sprays of phosphonic acid compounds. Crop Prot. 18:115–118.

Pegg, K. G., A. W. Whiley, and P. A. Hargreaves. 1990. Phosphonic (phosphorous) acid treatments control Phytophthora diseases in avocado and pineapple. Aust. Plant Pathol. 19:122–124.

Percival, G. C., K. Noviss, and I. Haynes. 2009. Field evaluation of systemic inducing resistance chemicals at different growth stages for the control of apple (*Venturia inaequalis*) and pear (*Venturia pirina*) scab. Crop Prot. 28:629–633.

Reuveni, M. and R. Reuveni. 1995. Efficacy of foliar sprays of phosphates in controlling powdery mildews in field-grown nectarine, mango trees and grapevines. Crop Prot. 14:311–314.

Reuveni, M., V. Agapov, and R. Reuveni. 1996. Controlling powdery mildew caused by *Sphaerotheca fuliginea* in cucumber by foliar sprays of phosphate and potassium salts. Crop Prot. 15:49–53.

Rodriguez, J. C., N. L. Shaw, and D. J. Cantliffe. 2007. Influence of plant density on yield and fruit quality of greenhouse-grown Galia muskmelons. HortTechnology 17:580–585.

Saindrenan, P., T. Barchietto, J. Avelino, and G. Bompeix. 1988. Effects of phosphite on phytoalexin accumulation in leaves of cowpea infected with *Phytophthora cryptogea*. Physiol. Mol. Plant Pathol. 32:425–435.

Smillie, R., B. R. Grant, and D. Guest. 1989. The mode of action of phosphite: Evidence for both direct and indirect modes of action on three *Phytophthora* spp. in Plants. Phytopathology 79:921–926.

Wang, Y. H., J. H. Huang, T. K. Lin, S. T. Wang, and C. H. Hsiao. 2004. Genetic studies and screening for resistance to powdery mildew (*Sphaerotheca fuliginea* race 1) in melon (*Cucumis melo* L.). J. Agric. Res. China 53:1–8. (in Chinese with English abstract)

Yamazaki, K. 1982. Nutrient Solution Culture. Pak-Kyo Co. Tokyo. 251 pp. (in Japanese)

中和亞磷酸對洋香瓜白粉病與果實品質之影響

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摘要

林楨祐、陳甘澍、黃雅穗、羅惠齡、洪爭坊。2016。中和亞磷酸對洋香瓜白粉病與果實品質之影響。台灣農業研究 65(3):261–268。

溫室甜瓜 (*Cucumis melo* L.) 為台灣高價值的水果之一，*Podosphaera xanthii* 引起的白粉病，可感染甜瓜的葉和藤蔓並造成品質和產量的下降。本研究比較不同中和亞磷酸和賽福座殺菌劑對設施內直立式洋香瓜栽培的影響，以兩種濃度 (1 g L^{-1} 和 2 g L^{-1}) 之中和亞磷酸，並搭配兩種噴施間期 (每 7 天 1 次和每 14 天 1 次)，於著果期間每 7 天評估各處理組的罹病程度，並於採收後調查果實鮮重、果肉厚度與總可溶性固形物。結果顯示在不同施用濃度下，每 7 天噴施中和亞磷酸對白粉病的防治效果與賽福座殺菌劑沒有顯著的差異 (P = 0.05)。然而，每 14 天噴施中和亞磷酸 1 g L^{-1} 則無法有效地降低白粉病罹病度。在各單一濃度下比較每 7 天或 14 天噴藥間期的影響，每 7 d 噴藥間期對白粉病罹病程度有較佳的控制效果。在果實品質方面，每 7 天噴施中和亞磷酸 1 g L^{-1} 和每 14 天噴施 2 g L^{-1} 皆顯著增加果實的鮮重。整體而言，施用中和亞磷酸對果肉厚度與可溶性固形物的含量並無顯著提升，僅在第 2 次的試驗中，每 14 天噴施中和亞磷酸 2 g L^{-1} 則有較佳的果肉厚度。因此，考量設施內洋香瓜果實品質的提昇和白粉病的控制，以每 7 天噴施中和亞磷酸 1 g L^{-1} 之處理應為較佳的選擇。

關鍵詞：洋香瓜、中和亞磷酸、白粉病、非農藥防治。

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研究報告

Pseudomonas viridiflava 引起之番茄髓壞疽病

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摘要

蔡佳欣、安寶貞、呂昀陞、陳美德、黃淑苓、洪挺軒。2016。*Pseudomonas viridiflava* 引起之番茄髓壞疽病。台灣農業研究 65(3):269–277。

2011 年於南投縣與花蓮縣地區少數番茄栽植田，發現部分植株出現生長緩慢及衰弱情形，病株莖部表面出現暗褐色塊狀病斑，縱切莖部發現其髓部組織出現褐色壞疽，病勢嚴重時整株萎凋。從罹病莖部壞疽組織分離純化出 1 種病原細菌，該細菌經接種番茄植株證實可引起相同病徵，並完成科霍氏法則，確認為該細菌所引起。該細菌經生理生化特性、Biolog 系統測試及 16S rDNA 序列分析，鑑定出此細菌為 *Pseudomonas viridiflava*，此為 *P. viridiflava* 在台灣造成番茄細菌性髓壞疽病之首次報導。以濾紙圓盤擴散法測試市售 10 種藥劑對該病原菌的抑制圈大小，結果顯示在 3 種有效測試濃度 (ai) 下對病原菌之抑制生長效果，以歐索林酸最佳。

關鍵詞：番茄、髓壞疽病、髓壞疽病菌、藥劑篩選。

前言

番茄 (*Solanum lycopersicum* L.) 為茄科 (Solanaceae) 茄屬 (Solanum)，為世界性的重要蔬菜之一，品種相當多樣。依行政院農業委員會之「農業統計年報」 (Council of Agriculture 2013)，台灣於 2012 年栽種番茄面積達 4,501 ha，每公頃收穫量約為 24,751 kg，主要產區在中南部，其中以嘉義縣種植面積最大。2011 年起在南投縣與花蓮縣等地區番茄栽植田，少數田區的植株出現異常的衰弱現象，病株生長緩慢、莖表面出現暗褐色塊狀病斑，其內部髓部組織褐化壞疽，果實發育遲緩及伴隨著落果現象。惟病害進程緩慢，罹病植株可維持數月不死亡，嚴重時發病株出現萎凋而影響果實品質及產量。將罹病番茄莖部之壞疽病組織於光學顯微鏡下觀察可見大量細菌自切口處泳出，因此推測可能為細菌所引起之病害。

由國內外文獻資料可知番茄的細菌性病害頗多，包括 *Ralstonia solanacearum* 引起之青枯病 (Hsu *et al*. 2002)，*Pectobacterium carotovorum* subsp. *carotovorum* 引起之細菌性莖腐病 (Hseu *et al*. 2003)，Xanthomonas 屬病菌 (*Xanthomonas perforans*, *Xanthomonas vesicatoria*, *Xanthomonas euvesicatoria*, *Xanthomonas gardneri*) 引起之細菌性斑點病 (Lue *et al*. 2010)，以及 Pseudomonas 屬病菌 (*Pseudomonas viridiflava*, *Pseudomonas cichorii*, *Pseudomonas mediterranea*, *Pseudomonas corrugata*) 引起之髓壞疽病 (Alippi *et al*. 2003; Aysan *et al*. 2004; Moura *et al*. 2005) 或細菌性葉斑病 (Tsai *et al*. 2014)。目前臺灣已被報導可感染番茄莖部並於維管束蔓延造成番茄植株萎凋之細菌性病害，有青枯病及細菌性莖腐病。因此，乃將田間罹病番茄莖部之壞疽組織，參考 Hseu *et al*. (2007) 及 Opina *et*

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al. (1997) 所述，以專一性引子進行番茄莖腐病及青枯病原細菌之 PCR 快速檢測。結果並未檢測出上述兩種病原細菌的感染，據此推測可能為其他的病原細菌所造成。

由於此病可造成番茄植株生長遲緩、易落果及嚴重時萎凋等各現象，若大量感染時將造成嚴重的經濟損失，因此本研究對此病之病因、病原菌特性及室內藥劑篩選進行探討，期以提供栽培及防治參考。

材料與方法

菌株來源

自南投縣與花蓮縣番茄發病田區採集具生長遲緩及莖部具褐化壞疽病徵之病株 (圖 1)，切取罹病莖部病健部組織以 1% 次氯酸鈉 (NaOCl) 漂洗 30 s 進行表面消毒，再以 3 次無菌水漂洗後，將組織切碎置入無菌水中震盪，以釋出組織液。之後，用移植環沾取懸浮液，以劃線平板方式塗於 NA (nutrient agar) (Difco Laboratories; Becton, Dickinson and Company, France) 培養基平板上，在 26℃定溫箱培養 2–3 d。待細菌長出單一菌落後，將其挑取至新的 NA 培養基平板，重複 3 次以純化細菌。

病原性試驗

接種試驗以市售番茄栽培品種「紅番」之供試植物，供試植物株高約 45–60 cm，種植於盆口直徑約 12 cm 之盆缽，以泥炭土、珍珠石及蛭石混合 (比例 2 : 1 : 1) 作為栽培介質。細菌菌株選取分離自南投縣之菌株 (編號為 TPN01 及 TPN02) 及分離自花蓮縣菌株 (編號 TPN03 及 TPN04)，合計共 4 分離株為供試菌株。接種原製備方法為，將供試細菌菌株培養於 NA 培養基平板，在 26℃培養 1 d 以後，以無菌水將細菌配置為懸浮液，並以分光光譜儀 (spectrophotometer, Bausch & Lomb, Bridgewater, NJ) 調整細菌懸浮液濃度至吸收

圖 1. 田間番茄髓壞疽病之病徵。(A) 髓部組織出現褐色壞疽。(B) 嚴重感染時呈深褐色且空心化。

Fig. 1. Symptoms of pith necrosis of tomato in the field. The pith of stem shows brown discoloration (A) and collapse in severe infected plant (B).

值 (A600) 為 0.3 (約 10^8 cfu mL^{-1}) 作為接種原。接種方法為先置 1 滴 10^8 cfu mL^{-1} 濃度之細菌懸浮液在番茄莖部，之後以滅菌之針頭於該處刺 1 小孔供病菌感染，以相同方法接種無菌水作為對照組。接種後植株套上透明塑膠袋保濕 48 h，之後除去塑膠袋，將植株放置在 26℃生長箱觀察，每 1 供試菌株接種 3 棵，並紀錄病徵發展情形，之後再從接種番茄之罹病莖部組織分離細菌，確認是否與原接種之細菌相同。

Biolog Identification System 鑑定細菌

以 Biolog 鑑定系統對上述 4 供試菌株進行分析，將供試菌株培養於 5.7% BUG™ Agar (Biolog Universal Growth Agar, Biolog Inc., Hayward, CA) 培養基培養 16–24 h，重複劃線培養於 BUG™ Agar 16–24 h，之後以 GN/GP-IF 緩衝液 (0.4% NaCl; 0.03% pluronic F-86; 0.01% gellan gum) 將細菌懸浮，並以波長 590 nm 之濁度計 (Turbidimeter, Biolog Inc.)，調整細菌懸浮液濃度至 52% T (Turbidity)。再將細菌懸浮液接種至 Biolog GN2 反應盤 (Biolog Inc. Hayward, CA) 中，每孔接種 150 μL 細菌懸浮液。將反應盤置於 30℃定溫箱培養 16–24 h，之後以光譜儀測讀，所得資料以 Biolog GN 資料庫 (Biolog 6.01 版) 比對分析，以鑑定該細菌種類。

生理生化特性測定

將 4 株供試菌株生理生化特性的測定結果，參考 Schaad *et al*. (2001) 所編著之「植物病原細菌鑑定實驗指南」進行下列生理生化測定，包括有革蘭氏反應 (Gram reaction)、對葡萄糖利用方式 (氧化/發酵試驗；O/F test)、在 King's B 培養基上產生螢光色素的測定、在結晶紫果膠 (crystal violet pectate) 培養基上測定果膠分解能力、在 YDC (yeast extract-dextrose-$CaCO_3$) 培養基上形成之菌落型態、37℃下之生長能力、果聚醣 (levan) 的生成、氧化酵素 (oxidase test) 測定、馬鈴薯軟腐測試 (potato soft rot)、精氨酸二水解酶 (arginine dihydrolase) 測定、菸草過敏性反應 (tobacoo hypersensitivity reaction) 測試、明膠 (gelatin) 液化能力測定，以及對 mannitol、cellobiose、sorbitol、trehalose、sucrose、meso-tartrate、D(-)-tartrate、D-arabinose、D-aspartate 的利用能力，以鑑定供試菌株分類地位。

菌株分子鑑定

選取由南投縣分離之 TPN02 及花蓮縣分離之 TPN03 菌株，於 NA 培養基培養後，參考 Wang *et al*. (1993) 之簡易方法並略為修改後進行核酸萃取。以滅菌牙籤沾取經純化後之單一菌落，放入 20 μL 之無菌水中懸浮，再加入 20 μL 之 0.4 N NaOH 震盪均勻後靜置 10 min。再加入 1 M Tris-HCl 40 μL 混合均勻後，取 20 μL 以無菌水稀釋 10× 製備為模板。之後以細菌 16S rDNA 之通用引子對 f8-27/r1510 (Lipson & Schmidt 2004) 進行聚合酵素連鎖反應 (polymerase chain reaction; PCR)，PCR 增幅後之 DNA 產物以 1.4% agarose 進行電泳分析，確定是否產生預期之 DNA 片段。所得之 PCR 產物以 TOPO TA cloning kit (Invitrogen Corporation, Carlsbad, CA) 進行 DNA 片段選殖，所得之選殖菌株經增殖後抽取質體，再以 PCR 及電泳分析確認選殖所得之片段為預估長度後，委由明欣生物科技有限公司 (Mission Biotech, Taipei, Taiwan) 進行定序後，再進行 DNA 序列比對。

藥劑感受性測定

選取 TPN02 細菌分離株，進行藥劑感受性試驗。供試藥劑種類包括 10 種市售藥劑，包括有抗生素類之鏈四環黴素 (streptomycin + tetracycline，10.0% SP，商品名為枯萎寧，全台公司)、鏈黴素 (streptomycin，12.5% SL，商品名為保安素，聯利公司)、嘉賜黴素 (kaugamycin，2.0% WP，商品名為菌友，世大化工)；含銅類藥劑之氫氧化銅 (copper hydroxide，53.8% WG，商品名為克菌多，台灣杜邦公司)；鹼性氯氧化銅 (copper oxychloride，70.0% WP，商品名為新必利丹，青山公司)；三元硫酸銅 (tribasic copper sulfate，27.12% SC，商品名為果太保，嘉濱貿易公司)；含鋅錳類藥劑之鋅錳乃浦 (mancozeb，80.0% WP，商品名為興農生-45，興農公司)；混合類藥劑之多保鏈黴素 (thiophanate methyl + strepto-

mycin，68.8% WP，商品名為克腐，大勝化工)；嘉賜銅 (kasugamycin + copper oxychloride，81.3% WP，商品名為加速黴素，世大化工) 及其他類藥劑之歐索林酸 (oxolinic acid，20.0% WP，商品名為金星，台灣住友公司)。供試藥劑濃度參考一般農友使用濃度調整設定為有效成分 40、200 及 1,000 mg L^{-1}。

測試方法以濾紙圓盤擴散法 (paper disc diffusion method) (Adaskaveg & Hine 1985)，測試供試菌株對上述 10 種藥劑在不同濃度之感受性。方法如下，製備細菌懸浮液並調整濃度約為 10^8 cfu mL^{-1}，取 0.1 mL 細菌懸浮液加入 6 mL 之水瓊脂 (Water agar) 中混合均勻，再將其覆於 NA 培養基平板上。將已稀釋成不同濃度的藥劑各 0.14 mL 滴入直徑 13 mm 之濾紙圓盤 (Whatman International Ltd.) 中，再將含藥之濾紙圓盤放在已經覆上含細菌水瓊脂之 NA 培養基平板上，並以滴入無菌水之濾紙圓盤作為對照組，每處理 3 重複。之後將各處理的培養基平板，放置在 26℃下定溫箱培養 48 h 後，測量抑制圈大小，以測定藥效。

結果

病原性試驗

將 4 株供試菌株 TPN01 與 TPN02 (分離自南投縣) 及 TPN03 與 TPN04 (分離自花蓮縣)，以穿刺法接種細菌懸浮液於供試紅番番茄植株莖部 3 wk 後，將接種之莖部縱切觀察。發現莖內髓部組織呈現褐色壞疽病徵，並從接種處沿維管束蔓延 (圖 2)。持續觀察所接種番茄植株生長緩慢，2–3 mo 後陸續開始出現萎凋現象，病程進展緩慢，與田間所見相同。從接種植株發病蔓延之壞疽病組織處，可再分離出細菌，該細菌經 Biolog 鑑定系統與生理生化特性分析結果與原接種細菌特性相同，接種無菌水之對照植株則無病徵出現。

生理生化測定

供試菌株 TPN01、TPN02、TPN03 及 TPN 04，生理生化特性測定結果 4 細菌菌株均為革蘭氏陰性 (Gram negative)。以氧化方式利用葡萄糖在 NA 培養時，菌落為淡金褐色；培養在 King’s B 時會產生螢光色素；培養於 YDC

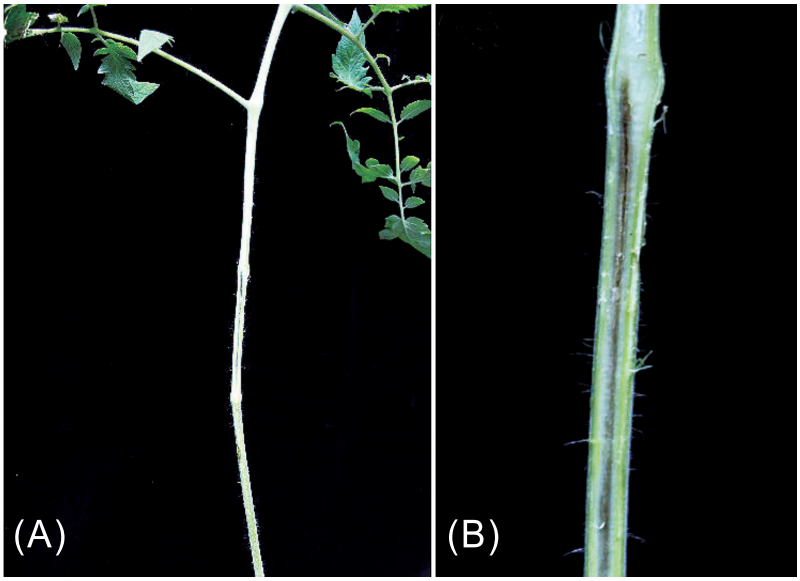

圖 2. 番茄人工接種分離到的細菌後呈現的病徵。(A)「紅番」番茄接種 3 wk 後出現髓部壞疽的病徵；(B) 髓壞疽病徵放大圖。

Fig. 2. Symptom appearance of the tomato inoculated with the isolated bacteria. (A) ‘Hong Fan’ cultivar showed pith necrosis symptom in 3 weeks after inoculation; (B) Close-up of the pith necrosis symptom.

培養基不產生黏稠狀菌落；培養於結晶紫果膠 (crystal violet pectate; CVP) 平板上時形成凹陷。又菌株不產生果聚醣 (levan)，不具氧化酶 (oxidase)；不具精氨酸二水解酶 (arginine dihydrolase)。當接種在馬鈴薯組織塊可使其軟腐，注射接種菸草葉片可誘導產生過敏性反應 (tobacoo hypersensitivity)。此外，可液化白明膠 (gelatin)，於 37℃下不生長。可利用 mannitol、sorbitol、meso-tartrate、D(-)-tartrate，不能利用 cellobiose、trehalose、sucrose、D-arabinose 及 D-aspartate，如表 1 所示。

Biolog system 鑑定結果

將供試菌株 TPN01–04 共 4 株，以 Biolog Identification System 分析 4 菌株對 95 種碳素源的利用情形，將試驗所得資料於 Biolog GN 資料庫 (Biolog 6.01 版) 比對，結果顯示各供試菌株均屬於 *P. viridiflava* (*syringae*)，相似值依序為 0.768、0.879、0.787 及 0.907。

分子鑑定結果

選取分離自南投縣之供試菌株 TPN02 與花蓮縣之供試菌株 TPN03，以細菌 16S rDNA 通用引子對 f8-27/r1510 進行 PCR 增幅，均可增幅出一約 1500-bp 之 DNA 片段。此 DNA 片段經選殖及定序後，菌株核酸序列 TPN02 (NCBI Accession KM357829) 及 TPN03 (NCBI Accession KM357829) 經 NCBI (National Center for Biotechnology Informa-

表 1. 番茄髓壞疽病菌之生理生化分析。
Table 1. Physiological and biochemical analysis on tomato pith necrosis pathogen.

Character	Strain from tomato	*Pseudomonas viridiflava*	*Pseudomonas syringae*[z]
Gram reaction	G (−)[y]	G (−)	G (−)
O/F[y] test	O	O	O
Fluorescent pigment on KB	+	+	+
Levan	−	−	+
Oxidase	−	−	−
Pectolytic activity	+	+	−
Arginine dihydrolase	−	−	−
Tobacco hypersensitivity reaction	+	+	+
Gelatin hydrolysis	+	+	V
Colonies yellow on YDC	−	−	−
Colonies mucoid on YDC at 30℃	−	−	−
Growth at 37℃	−	−	−
Utilization of:			
Mannitol	+	+	V
Cellobiose	−	−	−
Sorbitol	+	+	+
Trehalose	−	−	−
Sucrose	−	−	+
Meso-tartrate	+	+	+
D(-)-tartrate	+	+	−
D-arabinose	−	−	−
D-aspartate	−	−	−

[z] Data are from Schaad *et al*. (2001).
[y] G (−), Gram negative; O, oxidative; F, fermentation; +, postive; −, negative; V, 21–79% postive.

tion) 基因資料庫分析比對，比對結果 2 供試菌株均與 *Pseudomonas viridiflava* strain RM207.1a 及 KNOX249.1b (NCBI Accession Number:AY604845.1 及 AY604848.1) 之 16S rDNA 序列相同度達 99% 以上。

藥劑感受性測定

以濾紙圓盤擴散法，於 NA 培養基上，測定番茄髓壞疽菌株 TPN02 對不同藥劑在不同濃度下之感受性。10 種市售藥劑在 3 種有效成分濃度 40、200、1,000 mg L^{-1} 下，測試對該病菌生長之抑制效果。當藥劑有效成分濃度僅 40 mg L^{-1}，歐索林酸即產生抑制圈，抑制圈半徑 5.37 mm；當藥劑有效成分濃度為 200 mg L^{-1}，包含上述歐索林酸，鏈四環黴素亦出現抑制圈，半徑 8.83–2.80 mm；濃度為 1,000 mg L^{-1} 時，出現抑制圈之藥劑種類共計 3 種，為歐索林酸、鏈四環黴素及鏈黴素，半徑 1.87–10.20 mm。其餘 7 種藥劑包括嘉賜黴素、多保鏈黴素、嘉賜銅、鹼性氯氧化銅、氫氧化銅、三元硫酸銅及鋅錳乃浦，則均對供試菌株無抑制效果，各供試藥劑濃度於 NA 培養基形成之抑制圈直徑 (已扣除濾紙之大小) 如表 2 所示。

討論

2011 年在南投縣與花蓮縣番茄栽培區，發現番茄植株出現生長緩慢，莖部外表具暗褐色病斑，莖內髓部呈現壞疽病徵，且病勢嚴重時萎凋等現象之病株。此病害經病原菌分離及接種試驗確認，此問題為細菌所引起。該病原細菌續經測試為革蘭氏陰性；屬好氣性細菌，於 YDC (yeast extract-dextrose-$CaCO_3$) 平板培養時，菌落非黃色，不具黏稠狀；培養在 King's B 平板時會產生螢光色素；於 37℃不生長。將這些特性比對 Schaad *et al*. (2001) 所述資料，顯示該細菌屬於 *Pseudomonas* 屬。以 Biolog 鑑定系統測試 4 供試菌株，結果為 *Pseudomonas viridiflava* (*syringae*) 相似度在 0768–0.907 之間，顯示此病菌可能為 *Pseudomonas viridiflava* 或 *Pseudomonas syringae*。依 Schaad 等之細菌鑑定資料，上述 2 種細菌可由其他生理生化特性加以區分，因此進一步測試供試菌株之生理生化特性。發現該病菌可使馬鈴薯軟腐 (potato rot)，具果膠分解能力 (pectolytic activity)，不產生果聚醣 (levan)，可利用 D (-)-tartrate 之特性，而且與 *P. viridiflava* 相同但與 *P. syringae* 不同 (如表 1)，因此該菌與 *P. viridiflava* 較接近。此外，Lel-

表 2. 供試藥劑在不同濃度對番茄髓壞疽菌株 TPN02 之生長抑制效果。

Table 2. Growth inhibition of *Pseudomonas viridiflava* TPN02 by various agrochemicals in different concentrations.

	Inhibition zone (mm in diam.)		
Chemical	40 mg L^{-1}	200 mg L^{-1}	1,000 mg L^{-1}
Oxolinic (20% WP)	5.37 ± 0.30 a[z]	8.83 ± 0.18 a	10.20 ± 0.23 a
Streptomycin + Tetracycline (10% SP)	0 b	2.80 ± 0.06 b	5.83 ± 0.03 b
Streptomycin (12.5% SL)	0 b	0 c	1.87 ± 0.18 c
Thiophanate methyl + Streptomycin (68.8% WP)	0 b	0 c	0 d
Kasugamycin (2.0% WP)	0 b	0 c	0 d
Kasugamycin + Copper oxychloride (81.3% WP)	0 b	0 c	0 d
Copper hydroxide (77.0% WP)	0 b	0 c	0 d
Copper oxychloride (85.0% WP)	0 b	0 c	0 d
Tribasic copper sulfate (27.12% SC)	0 b	0 c	0 d
Mancozeb (80.0% WP)	0 b	0 c	0 d
LSD	0.08	0.17	0.27

[z] Mean ± standard error (n = 3). Means within each column followed by the same letter are not significantly different at 5% level by LSD test.

liot *et al*. (1966) 以 LOPAT (Levan-Oxidase-Potato rot-Arginine dihydrolase-Tobacco hypersensitivity) 測試法，將 Pseudomonas 屬細菌區分為 5 群，*P. syringae* 歸屬於第 1 群，*P. viridiflava* 歸屬於第 2 群，由本研究所分離之菌株測試結果為不產生果聚醣；不具氧化酶 (oxidase)；具有馬鈴薯軟腐能力；不具精氨酸二水解酶 (arginine dihydrolase)；可誘導菸草產生過敏性反應 (tobacco hypersensitivity) 的特性 (如表 1)，亦顯示所分離之病原細菌與 *P. viridiflava* 的特性相同，屬於 LOPAT 第 2 群，而與 *P. syringae* 不同 (LOPAT 第 1 群)。在分子鑑定上，分析供試菌株之 16S rDNA 序列與 *P. viridiflava* 相似度達 99% 以上。因此，綜合上述資料鑑定該菌為 *P. viridiflava*。在接種試驗，該菌可經由莖部傷口進入髓部感染及蔓延，造成髓壞疽病徵，證實具病原性，並可回分出相同病菌，完成科霍氏法則，確認該菌為病原菌。

P. viridiflava 寄主範圍廣泛，分布世界各地，可危害多種作物，如番茄 (tomato) (Alippi *et al*. 2003)、苜蓿 (alfalfa) (Heydari *et al*. 2014)、豌豆 (pea) (Martin-Sanz *et al*. 2010)、甜瓜 (melon) (Aysan *et al*. 2003)、蘋果 (apple) (Alimi *et al*. 2011)、羅勒 (basil) (Végh *et al*. 2012)、蘿蔔 (radish) (Shakya & Vinther 1989)、萵苣 (lettuce) (Ibrahim & Molan 2009) 等。該菌在國內尚未有危害番茄的報導，此為 *P. viridiflava* 感染番茄在台灣之首次紀錄。

由國外文獻可知，Pseudomonas 屬之多種病原菌 (*P. cichorii*、*P. corrugata*、*P. viridiflava*、*P. fluorescens* 及 *P. mediterranea*) 可造成番茄莖壞疽 (stem necrosis) 或髓壞疽 (pith necrosis) (Alippi *et al*. 2003; Aysan *et al*. 2004; Moura *et al*. 2005)，其中亦包括 *P. viridiflava*。本文依田間番茄病株與接種試驗之病株均具髓部壞疽之特殊病徵，與國外文獻描述相同 (Saygili *et al*. 2004)，因此將此病稱之為番茄髓壞疽病 (pith necrosis)。

依本次接種試驗顯示，此病菌可經由莖部小傷口感染番茄植株，因此病菌於田間，可能經植株修剪傷口入侵莖部感染。另外，國外研究指出，該病菌可殘存在番茄植株殘體、土壤、種子 (Yildiz *et al*. 2004) 及雜草 (Mariano & McCarter 1993)，因此於防治上應注意修剪工具的消毒、修剪後傷口的保護、及時清除病株、選擇非發病田種植、種子消毒及控制雜草。

在藥劑防治上，於室內篩選可抑制該病原細菌生長的藥劑，以供將來田間防治或種子消毒參考。試驗發現，全部 10 種供試藥劑中以歐索林酸抑制細菌生長之抑制圈顯著最大 ($P < 0.05$)，抗生素類藥劑之鏈四環黴素次之、鏈黴素再次之，然而含銅類藥劑包括氫氧化銅、三元硫酸銅及鹼性氯氧化銅等藥劑於所測試的 3 種濃度均無法抑制此菌的生長。由此顯示，田間存在對銅劑耐受度高之菌株，因此在田間應避免含銅類藥劑頻繁施用，以防止抗藥性問題的產生。

引用文獻

Adaskaveg, J. E. and R. B. Hine. 1985. Copper tolerance and zinc sensitivity of Mexican strain of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper. Plant Dis. 69:993–996.

Alimi, M., H. Rahimian, N. Hassanzadeh, M. T. Darzi, A. Ahmadikhah, A. Heydari, and G. M. Balestra. 2011. First detection of *Pseudomonas viridiflava*, the causal agent of blossom blight in apple by using specific designed primers. Afr. J. Microbiol. Res. 5:4708–4713.

Alippi, A. M., E. Dal Bo, L. B. Ronco, M. V. López, A. C. López, and O. M. Aguilar. 2003. Pseudomonas populations causing pith necrosis of tomato and pepper in Argentina are highly diverse. Plant Pathol. 52:287–302.

Aysan, Y., M. Mirik, A. Ala, F. Sahin, and O. Cinar. 2003. First report of *Pseudomonas viridiflava* on melon in Turkey. Plant Pathol. 52:800.

Aysan, Y., N. Yildiz, and F. Yucel. 2004. Identification of *Pseudomonas viridiflava* on tomato by traditional method and enzyme-linked immunosorbent assay. Phytoparasitica 32:146–153.

Council of Agriculture. 2013. 2012 Agricultural Statistics Yearbook. Council of Agriculture (COA). Taipei, Taiwan. 348 pp. (in Chinese)

Heydari, A., G. Khodakaramian, and D. Zafari. 2014. Occurrence, genetic diversity and pathogenicity charac

teristics of *Pseudomonas viridiflava* inducing alfalfa bacterial wilt and crown root rot disease in Iran. Eur. J. Plant Pathol. 139:299–307.

Hseu, S. H., C. Y. Lin, and T. C. Sung. 2003. Bacterial stem rot of tomato caused by *Erwinia carotovora* subsp. *carotovora*. Plant Prot. Bull. 45:257–262. (in Chinese with English abstract)

Hseu, S. H., H. Shentue, K. C. Tzeng, and C. Y. Lin. 2007. Development of specific primers for differential identification pathogen *Erwinia carotovora* subsp. *caratovora* and *Erwinia chrysanthemi*. Plant Pathol. Bull. 16:19–29. (in Chinese with English abstract)

Hsu, S. T., T. T. Chang, C. A. Chang, J. L. Tsai, and T. T. Tsai. 2002. List of Plant Diseases in Taiwan. 4th ed. Taiwan Phytopathological Society. Taichung, Taiwan. 386 pp. (in Chinese)

Ibrahim, Y. and Y. Molan. 2009. Occurrence of bacterial leaf spot disease on lettuce caused by *Pseudomonas viridiflava* in the Kingdom of Saudi Arabia. New Dis. Rep. 19:48.

Lelliott, R. A., E. Billing, and A. C. Hayward. 1966. A determinative scheme for the fluorescent plant pathogenic Pseudomonads. J. Appl. Bacteriol. 29:470–489.

Lipson, D. A. and S. K. Schmidt. 2004. Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. Appl. Environ. Microbiol. 70:2867–2879.

Lue, Y. S., W. L. Deng, Y. F. Wu, A. S. Cheng, S. T. Hsu, and K. C. Tzeng. 2010. Characterization of *Xanthomonas* associated with bacterial spot of tomato and pepper in Taiwan. Plant Pathol. Bull. 19:181–190.

Mariano, R. L. R. and S. M. McCarter. 1993. Epiphytic survival of *Pseudomonas viridiflava* in tomato and selected weed species. Microb. Ecol. 26:47–58.

Martin-Sanz, A., J. L. Palomo, M. Pérez de la Vega, and C. Caminero. 2010. First report of bacterial blight caused by *Pseudomonas viridiflava* on pea in Spain. Plant Dis. 94:128.

Moura, M. L., M. A. Jacques, L. M. Brito, I. M. Mourão, and J. Duclos. 2005. Tomato pith necrosis (TPN) caused by *P. corrugata* and *P. mediterranea*: Severity of damages and crop loss assessment. Acta Hort. 695:365–372.

Opina, N., F. Tavner, G. Hollway, J. F. Wang, T. H. Li, R. Maghirang, M. Fegan, A. C. Hayward, V. Krishnapillai, W. F. Hong, B. W. Holloway, and J. N. Timmis. 1997. A novel method for development of species and strain-specific DNA probe and PCR primers for identifying *Burkholderia solanacearum* (formerly *Pseudomonas solanacearum*). Asia Pac. J. Mol. Biol. Biotechnol. 5:19–30.

Saygili, H., Y. Aysan, F. Sahin, N. Ustun, and M. Mirik. 2004. Occurrence of pith necrosis caused by *Pseudomonas fluorescens* on tomato plants in Turkey. Plant Pathol. 53:803.

Schaad, N. W., J. B. Jone, and W. Chun. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd ed. American Phytopathological Society. St. Paul, Minnesota. 373 pp.

Shakya, D. D. and F. Vinther. 1989. Occurrence of *Pseudomonas viridiflava* in seedlings of radish. J. Phytopathol. 124:123–127.

Tsai, C. H., P. J. Ann, Y. S. Lu, M. D. Chen, S. L. Hwang, and Y. H. Peng. 2014. Occurrence of bacterial leaf spot of tomato caused by *Pseudomonas cichorii* in Taiwan. J. Taiwan Agric. Res. 63:143–150. (in Chinese with English abstract)

Végh, A., M. Hevesi, Zs. Némethy, and L. Palkovics. 2012. First report of bacterial leaf spot of basil caused by *Pseudomonas viridiflava* in Hungary. Plant Dis. 96:141.

Wang, H., M. Qi, and A. J. Cutler. 1993. A simple method of preparing plants samples for PCR. Nucleic Acids Res. 21:4153–4154.

Yildiz, H. N., Y. Aysan, F. Sahin, and O. Cinar. 2004. Potential inoculum sources of tomato stem and pith necrosis caused by *Pseudomonas viridiflava* in the Eastern Mediterranean region of Turkey. J. Plant Dis. Prot. 111:380–387.

Occurrence of Pith Necrosis of Tomato Caused by *Pseudomonas viridiflava* in Taiwan

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Abstract

Tsai, C. H., P. J. Ann, Y. S. Lu, M. D. Chen, S. L. Hwang, and T. H. Hung. 2016. Occurrence of pith necrosis of tomato caused by *Pseudomonas viridiflava* in Taiwan. J. Taiwan Agric. Res. 65(3):269–277.

In 2011 some infected tomato plants were noticed in several fields of Nantou and Hualien Counties. Dark brown blotch was observed in the surface of stem. Internally, pith and vascular tissues showed brown discoloration and necrosis symptom. The pathogenicity of the bacteria isolated from necrotic stem tissues was verified by Koch's postulates. The pathogen was further identified as *Pseudomonas viridiflava* based on physiological and chemical tests, Biolog identification, and 16S rDNA sequence analysis. This is the first report of pith necrosis of tomato caused by *P. viridiflava* in Taiwan. On screening the agrochemicals, oxolinic acid was the most efficient to inhibit the pathogen growth.

Key words: Tomato, Pith necrosis, *Pseudomonas viridiflava*, Agrochemical screening.

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研究報告

茭白矮化症之發生與防治

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摘要

黃晉興、安寶貞。2016。茭白矮化症之發生與防治。台灣農業研究 65(3):278–285。

2001 年在埔里發生一種茭白矮化的病害，發病面積約 500 ha，罹病株葉片變窄而短、葉片與葉鞘的角度常大於 45 度而葉領間距縮短，以致於外觀呈現矮化的病徵。有些發病植株於幼苗期即結筍，但所結之筍細小而無商品價值，這病害稱之「矮化症」。經由生長箱栽培試驗結果顯示，茭白正常植株經過長暗期 (16 h d^{-1}) 或長暗期 (16 h d^{-1}) + 暗期中斷 (1 h d^{-1}) 的處理，則呈現與田間相同的矮化病徵，矮化的病株經過長光期 (16 h d^{-1}) 或長光期 (16 h d^{-1}) + 光期中斷 (1 h d^{-1})，則恢復正常生長。田間夜間照光試驗區分為照光 0、4、8 及 12 h 的處理，60 d 後矮化症發病率分別為 45.3、3.5、0 與 0%，株高分別為 127.0、158.5、171.2 與 186.4 cm，鮮重分別為 2.5、3.2、3.8 與 4.3 kg $plant^{-1}$。茭白於正常期 (1 月) 種植與提早種植 20 d 或 40 d 之處理，發病率分別為 5.4、14.9 與 55.3%，三者皆有顯著差異 ($P < 0.05$)，而這三時期種植予以夜間照光 8 h，則發病率皆低於 1.1%。由上述結果，顯示矮化症是茭白植株於長夜季節的生理反應，提早於冬季中期前種植會使病害嚴重，夜間照光可防治矮化症並能促進植株生長。

關鍵詞：茭白、矮化症、夜間照光、防治。

前言

茭白 (*Zizania latifolia* Turcz.) 為喜水、多年生宿根性草本植物，在分類上屬於禾本科 (Gramineae) 菰屬 (Zizania)，其英名為 water bamboo、water oat、coba 或 Manchurian wild rice (Leu *et al*. 1977; Terrell & Batra 1982; Ann *et al*. 2006; Chang *et al*. 2008)。當茭白莖部受茭白黑穗菌 (*Ustilago esculenta* P. Henn.) 感染後，細胞受黑穗菌產生之生長激素 (IAA) 及細胞分裂素 (Cytokinin) 刺激 (Liu & Kuo 1980; Hung *et al*. 2006)，形成肥大紡緟形可食用的菌癭，即為茭白筍，俗稱水筍、腳白筍或美人腿 (Lin 2005)。已受茭白黑穗菌感染的植株，其無性繁殖的下一代芽體體內即有茭白黑穗菌的存在，而無茭白黑穗菌感染而會開花的植株，其下一代也會開花 (Yang 1976; Chung & Tzeng 2004)。

根據行政院農業委員會農糧署 (統計室) 的統計資料顯示，台灣茭白筍的栽培面積計約有 1,000 ha，南投縣為最大栽培區，約占 85%，栽培地多集中在埔里鎮。當地茭白種植的方式採用一年生栽培，每年須重新整地，在前年 12 月上旬育苗而次年 1 月定植於水田，約再 3 mo 後開始採收，栽培期為 1–10 月 (Lin 2005)。

埔里的茭白定植於本田後，有時會出現植株矮化、叢生而無法正常生長的病害，稱為「矮化症」，有人認為是營養元素缺乏或是病毒病害，但真正原因不明。然而在 2001 年 1 月，埔里地區所栽種的茭白植株出現大規模的矮化症，估計約有500 ha茭白田發生此病害 (Huang *et al*. 2009)，農民採取的應對方法為廢耕而重新種植。當年的田間觀察發現在大面積茭白矮化的田區中，有一部分在路燈下的茭白植株卻

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生長正常，而此路燈主要用於夜間照明，顯示夜間照光可能會影響矮化症。本研究探討此矮化症之病因，並應用夜間照光以研發防治方法。

材料與方法

茭白栽培

盆缽栽培：本文使用之供試茭白品種如無特別說明均為「早生青殼」，植株來源分別為：(1) 自於南投縣埔里鎮田間取回苗齡 60 d 之茭白植株 (種莖育苗 30 d + 本田 30 d)，從整叢植株取出部分分蘗苗 (含根) 移植至盛有 2.5 L 農試所田土與 2.5 L 地下水之圓柱形桶中 (直徑 24 cm，高度 30 cm)；(2) 取上一季留下的茭白種莖，種植於盛有上述農試所田土與地下水之圓柱形盆中，於 24℃ (光照 12 h d^{-1}，光照強度為 51.7–70.3 μmol m^{-2} s^{-1}) 生長箱培養 60 d 備用，每盆種植 1 株 (約有 3–5 枝分蘗苗)。上述 2 種茭白植株做為本試驗之盆栽供試植株。

田間栽培：田間試驗地點為南投縣埔里鎮，所使用的種莖來自前一期作的茭白結筍株，自 10 月中旬將田水排乾，於 11 月將地下部枝葉割除，留下長約 15–20 cm 之莖部，再將帶有莖部之植叢基部 (含土) 挖出，取下帶有芽體的基部莖段 (長 10–20 cm)，經太陽曝曬數日乾燥後，再置於 2℃冷藏庫備用，視為種莖。慣行農法為 12 月上旬育苗 1 個月而於次年 1 月上旬移植於本田，育苗時於田地築土畦 (高約 20 cm)，保持畦溝有水及畦土潮濕，每段種莖約可長出 3–10 個側芽或分蘗視為 1 株，育苗約 30 d 後，苗高 30–45 cm 即可移植於本田，每公頃約種植 8,000 株 (叢)。茭白植株由母莖節可發育數十分枝而形成叢狀，本田維持浸水的狀態，視植株生長逐漸調高水位，於結筍期達到最高水位約 60–90 cm 深。

茭白矮化症病害調查

2001 年赴南投縣埔里鎮茭白筍栽培田調查矮化症的發生率，逢機選取數塊栽培田，每塊田選取 100 株 (叢) 茭白植株，將植株矮化、葉片變窄而葉領間距縮短視為發生矮化症 (圖 1)，調查矮化症之發病率。此外，於當年 3–5 月赴新北市三芝區與宜蘭縣礁溪鎮，調查是否有矮化症的病害 (茭白品種為「赤殼」)。往後數年並再赴南投縣埔里鎮調查茭白矮化症。

長光期或長暗期處理對茭白生長之影響

2001 年自埔里田間取回起苗齡 75 d 之茭白植株 (種莖育苗 30 d + 本田 45 d)，該茭白苗為上期作結筍株 (受茭白黑穗菌感染) 的種莖育苗而成，試驗植株區分為矮化症病株與正常植株，如前述分缽栽培，將供試植株置於生長箱中 (日/夜溫 24℃/20℃光照強度為 51.7–70.3 μmol m^{-2} s^{-1})，進行 4 個處理：A 為正常株長光期處理 (照光 16 h d^{-1})、B 為正常株長暗期處理 (黑暗 16 h d^{-1})、C 為矮化症病株長光期處理、D 為矮化症病株長暗期處理。每一處理 3 重複 (盆)，於種植 28 d 後記錄矮化症之發病情形，並將植株置於溫室中 (溫度 21–28℃，照光約 12 h d^{-1})，42 d 後 (3 月中旬–4 月下旬) 觀察並記錄茭白結筍的情形。

另外，自埔里田間取回茭白結筍株 (受茭白黑穗菌感染) 與開花株 (無茭白黑穗菌感染) 之種莖，於前述生長箱中育苗 2 mo 後，如前述盆缽栽培，將供試植株置於生長箱中 (日/夜溫 24℃/20℃光照強度為 51.7–70.3 μmol m^{-2} s^{-1})，進行 4 個處理：A 為長光期處理 (照光 16 h d^{-1})、B 為長光期 + 光期中斷處理 (光-暗-光-暗期為 8-1-8-7 h d^{-1})、C 為長暗期處理 (黑暗 16 h d^{-1})、D 為長暗期 + 暗期中斷處理 (暗-光-暗-光期為 8-1-8-7 h d^{-1})。每一處理 5 重複 (盆)，於處理 28 d 後記錄矮化症之發病情形。

田間夜間照光對茭白矮化症之影響

2001 年 3 月於南投縣埔里鎮的茭白栽培田，進行田間夜間照光對茭白矮化症影響之初步試驗。選取發生輕微茭白矮化症而苗齡約 30 d 的茭白田區 (「早生青殼」品種)，利用日光燈施以夜間照光 6 h (18:00–24:00)，方法為架設 40 W 的白色日光燈 (台灣東亞 FL40D) 於 3 m 高的支柱上 (圖 2E)，每 3 m 一盞，以田區另一端以夜間不照光之植株為對照組，30 d 後計算距離田埂 5 排的茭白植株共 100 叢植

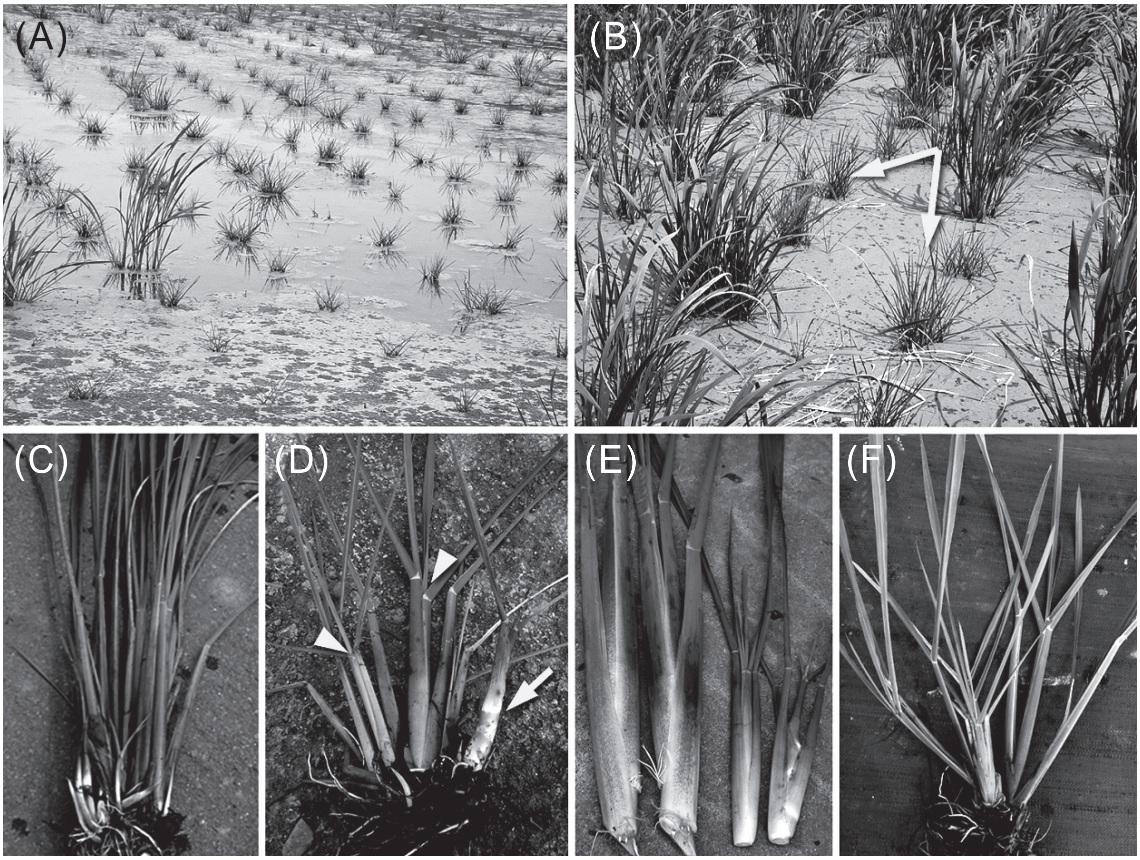

圖 1. 茭白筍矮化症的病徵。(A)、(B) 南投縣埔里鎮的茭白發生大規模矮化症，發病植株葉片變細小、矮化 (如箭號所示)；(C) 正常植株高大且葉片直立；(D) 發病植株矮小、葉片短小、葉片與葉鞘角度常大於 45 度且葉領間距縮短 (如箭頭所示)，有些發病植株於幼苗期即結筍 (如箭號所示)；(E) 罹病株所結之筍 (右 1 與 2) 較正常株 (左 1 與 2) 小；(F) 未感染黑穗菌的茭白開花株苗在冬天亦會在田間出現矮化症。

Fig. 1. Symptom of stunting disease of water bamboo. (A) and (B), large area of stunting water bamboo plants at Puli Township, Nantou County in January 2001. The diseased plants showed thinning leaves and stunting symptom (arrow). (C) The healthy plants were tall with upright leaves. (D) The diseased plants showed symptoms that the angles between leaf blade and leaf sheath were usually larger than 45°, the leaf collar distances were shorten (arrow head), and some edible galls (arrow) formed in the stunting seedling. (E) The size of the edible galls of the diseased plant (right-hand side) was smaller than those of the healthy plant (left-hand side). (F) The seedlings of smut-uninfected plants also showed stunting symptom in the field in winter.

株之矮化症發病率，行株距約 105 cm。

2002 年 12 月下旬自茭白苗移植於田間起，再測試夜間照光時數對茭白矮化症與植物生長之影響。方法為架設 20 W 的白色日光燈 (台灣東亞 FL20D) 於 2 m 高的電纜線上 (圖 2F)，每 3 m 一盞，自 18:00 起，夜間照光的時數分別為 4、8 及 12 h，以夜間不照光為對照組，共 4 個處理，試驗區採完全逢機設計 (CRD)，每小區 50–60 叢植株，3 重複，每個重複間隔約 3 m，60 d 後計算所有植叢矮化症發病率，並每重複任取 10 株測量株高與地上部枝葉鮮重。

種植時間與夜間照光對茭白矮化症之影響

由田間觀察顯示，提早種植之茭白發生的矮化症較嚴重，為了確認此現象，2003 年 12 月起於田間栽培茭白，分成提早 40 d (12 月

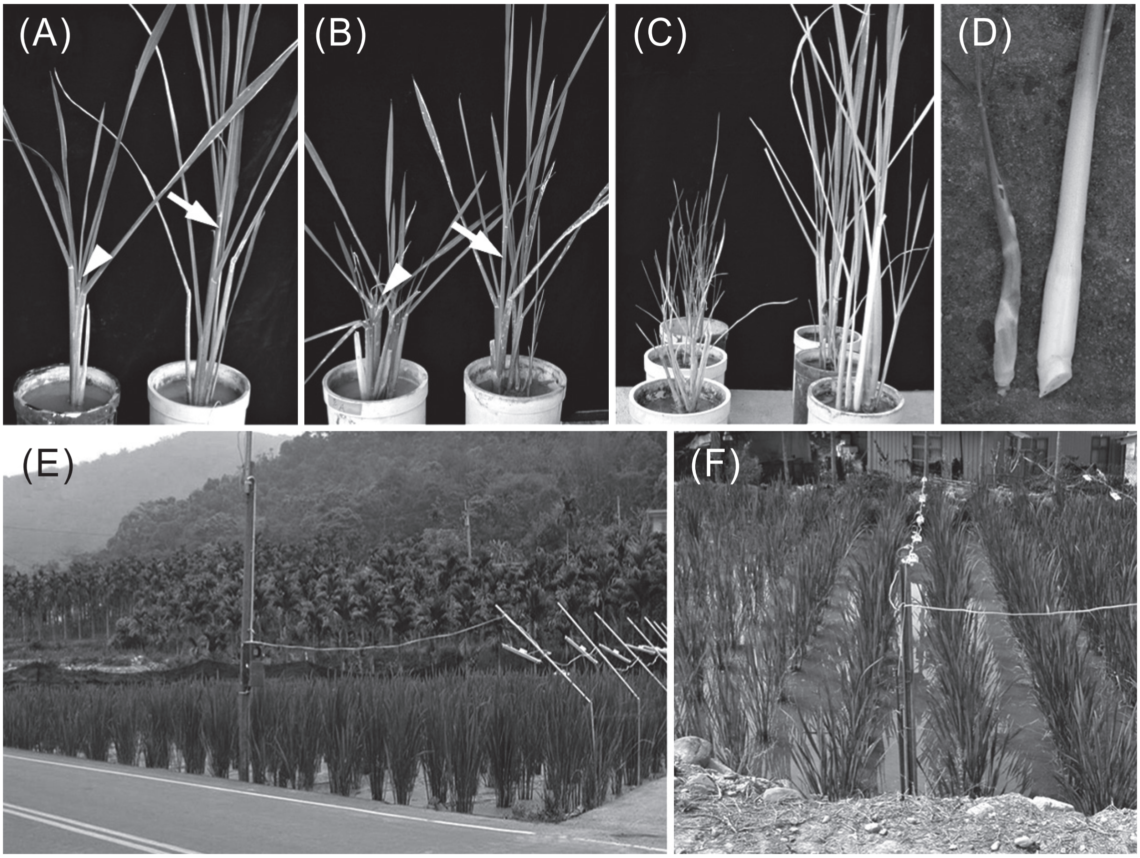

圖 2. 利用延長光期防治茭白筍矮化症。原本發生矮化症之植株 (A) 與原本正常的植株 (B) 於生長箱內經長暗期處理 (16 h d^{-1}) 4 wk 後葉領間距縮短 (如箭頭所示)，經長光期處理 (16 h d^{-1}) 則葉領間距拉長 (如箭號所示)；(C)、(D) 經長暗期處理的植株於溫室 6 wk 後產生短小的茭白筍體 (左) 而經長光期處理的植株則能產生正常的茭白筍 (右)；(E)、(F) 以日光燈進行田間茭白栽培長光期處理。

Fig. 2. Prolong light period for controlling stunting disease of water bamboo. The original normal plants (A) and the stunting plants (B) showed stunting symptom (arrow heads) after long-dark-period treatment (8 h d^{-1}), or showed elongation of leaf collar distance (arrows) after long-light-period treatment (16 h d^{-1}) in growth chambers. (C) and (D), the long-dark-period treated plants produced shortened edible gall (left), but the long-light-period treated plants produced normal edible gall (right) in greenhouse for 6 weeks. (E) and (F), using fluorescent lamps to prolong lighting at night for controlling stunting disease of water bamboo in the field.

2 日)、提早 20 d (12 月 22 日) 與正常期移植 (2004 年 1 月 11 日)。自 18:00 起夜間照光 8 h，方法為架設 20W 的白色日光燈於土面 3 m 高的電纜線上 (圖 2F)，每 2 m 一盞，以夜間不照光為對照組，共 6 個處理。試驗區採完全逢機設計 (CRD)，每小區 50–60 叢植株，3 重複，每個重複間隔約 3 m，於種植 60 d 後停止照光，計算所有植叢矮化症發病率，行株距約 105 cm。

結果

茭白矮化症之發生

2001 年 2 月南投縣埔里鎮茭白植株發生大規模矮化的病害 (圖 1A、B)，以目測估計發病面積約 500 ha，田區的發病率由 20–99% 不等，但大多數發病率皆超過 80%，以致多數農民放棄此次茭白而重新種植。由於發病植株之葉片變窄而短，葉片與葉鞘角度常大於 45 度，

而葉領間距縮短，以致於外觀呈現矮化的病徵 (圖 1C、1D)。有些發病植株於幼苗期即結筍，但病株所結之筍較健康株小很多 (圖 1E)，故這些提早結成的茭白筍無商品價值。2001 年新北市三芝區與宜蘭縣礁溪鎮所栽培的茭白植株並未發生矮化的病害。爾後數年調查南投縣埔里鎮茭白矮化的病害，部分田區仍出現矮化的病徵，發病率 5–50%，通常是提早栽培的田區才會發生較嚴重的矮化的病徵，惟未如 2001 年大規模發生的情形。此外，在田間亦可觀察得未感染黑穗菌的茭白開花株在冬天亦會出現矮化症 (圖 1F)。

長光期或長暗期處理對茭白生長之影響

自田間取回茭白正常植株與發生矮化的病株，置於長光期或長暗期處理的生長箱中，28 d 後，原本正常植株於長暗期處理呈現葉領間距縮短，而新葉高度較老葉低的矮化病徵，而長光期處理則葉領間距更拉長，植株高度更高，生長更旺盛 (圖 2A)。原本矮化病株於長暗期處理呈現更嚴重的矮化病徵，而長光期處理則葉領間距拉長，新葉長度較老葉長 (圖 2B)。將上述長暗期處理後的植株 (原正常植株或原矮化病株) 與長光期處理後的植株 (原正常植株或原矮化病株)，置於溫室 42 d 後，長暗期處理後的植株生長不良、矮化而結筍細小，而長光期處理後的植株生長較茂盛而結筍較粗大 (圖 2C、2D，表 1)。

自田間取回茭白結筍株 (受茭白黑穗菌感染) 與開花株 (無茭白黑穗菌感染) 之種莖育成苗齡 2 mo 的植株，於經過 28 d 長暗期處理或長暗期 + 暗期中斷處理皆呈現矮化的病徵，而經過 28 d 長光期的處理 (照光 16 h d^{-1}) 或長光期 + 光期中斷處理則不呈現矮化的病徵 (表 2)。

田間夜間照光對茭白矮化症之影響

由田間初步試驗顯示，冬春季夜間照光 6 h 可預防茭白矮化症，處理區皆正常且無矮化症植株，而夜間無照光的對照區則有 25% 的發病率 (圖 2E)。若將夜間照光的時數區分為 4、8 及 12 h 處理，夜間照光時間越長則矮化症的發病率越低，發病率分別為 3.7、0 與 0%，與夜間無照光的對照組發病率 45.3% 有顯著差異 (P < 0.05)；而株高與鮮重隨著夜間照光時間越長則株高越高，且鮮重越重，株高分別為 158.5、171.2 與 186.4 cm，與夜間無照光的對照組株高 127.0 cm 有顯著差異 (P < 0.05)；茭白植株枝葉的鮮重分別為 3.2、3.8 與 4.3 kg $plant^{-1}$，與夜間無照光的對照組株高 2.5 kg $plant^{-1}$ 有顯著差異 (P < 0.05) (表 3)。

種植時間與夜間照光對茭白矮化症之影響

茭白若提早種植則矮化症較嚴重，提早 40 d、提早 20 d 與正常期移植之處理，矮化症發病率分別為 55.3、14.9 與 5.4%，三者皆呈顯著差異 (P < 0.05)。而這三時期種植並予以夜間照光 8 h，發病率則僅為 1.1、0.7 與 0% (表 4)。

討論

2001 年的田間觀察發現於大面積茭白矮

表 1. 生長箱內長光期或長暗期處理對茭白生長之影響。

Table 1. Effect of long light period or long dark period on growth of normal and stunted water bamboo plants in growth chambers.

Original plant	Lighting treatment[z]	Stunting	Edible gall
Normal	Long light period[y]	No	Normal
	Long dark period[x]	Yes	Abnormal
Stunted	Long light period	No	Normal
	Long dark period	Yes	Abnormal

[z] In 24℃/20℃ (day/night) growth chamber with light intensity 51.7–70.3 μmol m^{-2} s^{-1}.
[y] Light period 16 h d^{-1}.
[x] Light period 8 h d^{-1}.

表 2. 生長箱長光期或長暗期與光或暗期中斷處理對感染或未感染黑穗菌之茭白植株生長之影響。

Table 2. Effect of long light period or long dark period or with light or dark interruption on growth of water bamboo plants (in growth chambers) which were infected smut fungi or not.

Plant	Lighting treatment[z]	Stunting
Smut infected	Long light period[y]	No
	Long light period + light interruption[x]	No
	Long dark period[w]	Yes
	Long dark period + dark interruption[v]	Yes
No smut infected	Long light period	No
	Long light period + light interruption	No
	Long dark period	Yes
	Long dark period + dark interruption	Yes

[z] In 24℃/20℃ (day/night) growth chamber with light intensity 51.7–70.3 μmol m^{-2} s^{-1}.
[y] Light period 16 h d^{-1}.
[x] Light-dark-light-dark period was 8-1-8-7 h d^{-1}.
[w] Dark period 16 h d^{-1}.
[v] Dark-light-dark-light period was 8-1-8-7 h d^{-1}.

表 3. 田間夜間照光時間長短對茭白矮化症與生長之影響。

Table 3. Effect of night lighting duration on the stunting disease and growth of water bamboo in the field[z].

Night lighting (h)[y]	Stunting ratio (%)	Plant height (cm)	Fresh weight (kg $plant^{-1}$)
0	45.3 a[x]	127.0 c	2.5 c
4	3.7 b	158.5 b	3.2 b
8	0.0 b	171.2 ab	3.8 ab
12	0.0 b	186.4 a	4.3 a

[z] Experiment period 60 days from December 10, 2002.
[y] Night lighting began at 18:00.
[x] Means within each column followed by the same letter are not significantly different at $P < 0.05$ according to the Fisher's least significance difference (LSD) test.

表 4. 種植時期 (2003–2004) 與夜間照光對茭白發生矮化症之影響。

Table 4. Effect of planting period (2003–2004) and night lighting on the stunting ratio of water bamboo.

Planting date	Lighting at night[z]	Stunting ratio (%)
Dec. 2, 2003	No	55.3 a[y]
	Yes	1.1 c
Dec. 22, 2003	No	14.9 b
	Yes	0.7 c
Jan. 11, 2003	No	5.4 c
	Yes	0.0 c

[z] Night lighting began at 18:00 for 8 h d^{-1} for 60 days.
[y] Means within each column followed by the same letter are not significantly different at $P < 0.05$ according to the Fisher's least significance difference (LSD) test.

化的田區中，有一部分在路燈下的茭白植株卻生長正常，顯示可能夜間照光可能會影響矮化症。由本研究的生長箱試驗結果顯示，正常的植株在數週的長暗期處理之後皆會產生矮化症的病徵，而矮化症的病株經由長光期處理後，則能恢復正常生長 (表 1、圖 2)。在田間，越早在冬季長夜時節種植則矮化症的發生率越高 (表 4)，顯示此病害乃是「早生青殼」品種茭白

植株在長夜季節的生理反應。2001 年以前，埔里栽培的「早生青殼」品種茭白也偶爾會發生矮化症，通常是在初春時期茭白筍苗移植至本田後出現，多僅是零星發生 (圖 2B)，這可能與植株個體間的生理差異有關。至於為何在 2001 年會大發生，目前尚無合理的解釋。

埔里茭白主要的栽培品種是「早生青殼」品種，原本的栽培期從 1 月 1 日左右開始至 10 月 15 日左右結束，4–6 月是第 1 次豐產期，8–10 月是第 2 次豐產期，每年 11–12 月是休耕期，這是數十年來的慣行農法 (Lin 2005)。在第一次豐產期茭白植株有 7–8 葉的展開葉時期所結的筍大小被認為是正常的，但是在 10 月採收末期時，茭白幼枝在 3–5 葉即紛紛結筍，筍體越來越小，而且爾後植株即不易高大，所以農民認為茭白生長期結束的時節到了，自然而然在 11–12 月休耕。由本研究的結果顯示，在 11–12 月的長夜季節，茭白植株矮化是一種自然的現象，因為無論有或無茭白黑穗菌感染的植株，在長暗期處理皆會產生矮化症的病徵，即使是未感染黑穗菌的茭白開花株在冬天田間亦會出現矮化症。因此，農民自動休耕，若不休耕而提早在 12 月初移植到田間則茭白矮化的機率大增。然而慣行栽培期在 12 月上旬開始育苗，田間茭白育苗期卻很少有矮化症的發生，顯示長夜對育苗期生長影響較小，原因尚不得而知。

由本研究的結果顯示，無論有或無茭白黑穗菌感染的茭白植株，長光期處理即使光期有中斷，植株仍不會矮化，而長暗期處理即使有暗期中斷，植株仍會矮化。而夜間補光的時間越長則生長越茂盛，這顯示可能是因為光合作用的時間增加而有助生長有關。其他相關原因，則仍待深入研究探討。

引用文獻

Ann, P. J., J. H. Huang, I. T. Wang, and W. H. Ko. 2006. *Pythiogeton zizaniae*, a new species causing basal stalk rot of water bamboo in Taiwan. Mycologia 98:116–120.

Chang, T. L., I. Z. Chen, C. N. Chang, and S. F. Hung. 2008. Microscopy studies of the symbiotic relationships between coba (*Zizania latifolia* Turcz.) and *Ustilago esculenta* P. Henn. Acta Hort. 774:443–447.

Chung, K. R. and D. D. Tzeng. 2004. Nutritional requirements of the edible gall-producing fungus *Ustilago esculenta*. J. Biol. Sci. 4:246–252.

Huang, J. H., P. J. Ann, and Y. S. Lin. 2009. Stunting disease, harvesting periods regulation and basal stem rot control of water shoots. Plant Pathol. Bull. 18:70–71. (in Chinese with English abstract)

Leu, L. S., H. C. Yang, and S. K. Sun. 1977. Preservation of wateroat, a crown gall of *Zizania latifolia* induced by *Ustilago esculenta*. Plant Prot. Bull. 19:238–244. (in Chinese with English abstract)

Lin, T. C. 2005. Water bamboo. p.271–276. *in*: Taiwan Agriculture Encyclopedia (Crop edition). 3rd ed. Council of Agriculture, Executive Yuan. Taipei, Taiwan. 4700 pp. (in Chinese)

Liu, S. D. and M. S. Kuo. 1980. Studies on the smut of vegetable wild rice, *Zizania latifolia*, cuased by *Ustilago esculenta*. IV. The extraction and identification of plant hormones from fungous gall and culture filtrate. Bull. Natl. Pingtung Inst. Agric. 21:106–114. (in Chinese with English abstract)

Terrell, E. E. and L. R. Batra. 1982. *Zizania latifolia* and *Ustilago esculenta*, a grass-fungus association. Econ. Bot. 36:274–285.

Yang, H. C. 1976. Studies on Storage of Wateroat and Pathological Histology and Its Causal Fungus, *Ustilago esculenta* Henning. Master thesis, Department of Plant Pathology, National Chung Hsing University. Taichung, Taiwan. 72 pp. (in Chinese with English abstract)

Occurrence and Control of Stunting Disease of Water Bamboo

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Abstract

Huang, J. H. and P. J. Ann. 2016. Occurrence and control of stunting disease of water bamboo. J. Taiwan Agric. Res. 65(3):278–285.

In spring 2001, a kind of stunting disease of water bamboo (*Zizania latifolia*) occurred in about 500 hectares of farmland in Puli Township, central Taiwan. The leaf blades of the diseased plants were short and narrow, the distances between leaf collars were shortened, and the angles of the blades and sheaths were greater than 45 degrees. These symptoms caused the appearance of the plants looked dwarfing. Some of the diseased seedlings produced small smut galls, but the edible galls were small and valueless. Results from cultivation test in growth chamber showed that the normal plants were stunted with the same stunting symptoms in the fields after the treatments of long dark period (16 h d^{-1}) or long dark period with dark interruption (1 h d^{-1}); whereas, the stunted plants were back to normal growth after the treatments of long light period (16 h d^{-1}) or long light period with light interruption (1 h d^{-1}). Field trials were conducted with different night-lighting time including 4 treatments, lighting for 0, 4, 8, and 12 h, respectively. The disease incidences were 45.3, 3.5, 0, and 0%, respectively, the plant heights were 127.0, 158.5, 171.2, and 186.4 cm, respectively, and the shoots fresh weights were 2.5, 3.2, 3.8, and 4.3 kg $plant^{-1}$, respectively. In the field test of beginning planting on the normal date (January 10th) or earlier by 20 or 40 days, the disease incidences were 5.4, 14.9, and 55.3%, respectively, with significant difference. By night-lighting for 8 h d^{-1} on these three treatments for 60 days, the disease incidences were all lower than 1.1%. The above results indicated that this stunting disease was a normal plant physiological response of water bamboo during the long-night season. Early planting before the mid-winter will enhance the disease, and nighttime illumination could prevent this disease and promote plant growth.

Key words: *Zizania latifolia*, Stunting disease, Night lighting, Control.

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氣候變遷災害、地區資本與原住民部落農業生產之關聯性

王俊豪 [1,*]

摘要

王俊豪。2016。氣候變遷災害、地區資本與原住民部落農業生產之關聯性。台灣農業研究 65(3):286–295。

有鑑於氣候變遷引發的極端天氣事件，已嚴重威脅高山原住民生活與部落農業生產，然而現有文獻中較缺乏原住民農業生產與氣候災害關聯性之量化實證研究。本研究從地域競爭力的角度，主要目的在於分析氣候異常現象與災害事件，對於高山地區農業經營的影響結果，及部落如何運用地區資本因應氣候變遷對農業生產的衝擊。研究資料來源為 2013 年新竹縣尖石鄉泰雅族部落的問卷調查，有效樣本 114 份。資料分析方法採用最小平方法的多元迴歸模型，分析部落地區資本存量、氣候變遷災害對於部落農業生產的影響結果。重要的研究發現，農作物品質降低和作物生長條件遭破壞，是氣候異常現象對於部落農業生產最為嚴重的衝擊。此外，部落地區資本存量愈高，將會顯著降低與減緩「氣候異常影響農業生產的嚴重性」，然而不同面向地區資本的影響效應不一，其中以部落社會資本因應「氣候異常影響農業生產」的減損效應最高；相對的，經濟資本則無顯著的影響結果。政策建議有關單位應以厚實原住民部落的社會資本，作為部落農業因應氣候異常衝擊的首要調適策略，諸如維持泰雅族部落的傳統生活方式與社會網絡，透過部落互助組織與共享價值來減緩氣候風險對農業生產的影響。

關鍵詞：原住民部落、氣候災害、地域競爭力、地區資本。

前言

「傾聽原住民的聲音」與「氣候正義」為 2009 年聯合國氣候變遷框架公約 (United Nations Framework Convention on Climate Change; UNFCCC) 的重要議題之一，其主要在於關切原住民部落在全球氣候變遷衝擊下的處境與角色，特別是對抗全球暖化時的資源使用與風險分配之公平性問題 (UNFCCC 2009)。由於台灣處於特殊的地理與地質環境，長年來深受地震、颱風、水患與土石流等天然災害之威脅，特別是高山原住民部落生存權維護的與生計產業發展上，近年則深受因全球暖化所造成的極端天氣災害影響，如豪大雨頻率與強度增加，乾旱次數增加或時間延長，季節降雨量的大幅變化，熱浪或寒流的溫度型災害，以及颱風、豪雨伴隨著洪水、土石流和山崩的複合型災害等 (Lin 2009)。這些天然災害不僅直接威脅原住民部落的生命安全與生活家園，同時不斷發生的極端天氣事件，也無法避免地衝擊著高山地區農業的永續發展，進而可能導致高山原住民部落賴以維生的生計來源，成為氣候變遷下的環境犧牲品。有鑑於此，本研究擬從社會發展的面向，審視位於石門水庫集水區上游原住民部落農業發展的困境與可能性，探討氣候變遷引發的氣候異常現象與氣候災害事件，對於高山地區農業經營的影響程度，以及泰雅族部落運用地區資本因應氣候變遷對農業生產的衝擊。

氣候變遷引發的極端天氣異常事件與自然生態的不可逆改變，已明顯威脅高海拔地區與社經弱勢會群體，特別對於高度依賴自

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然資源的偏遠地區原住民和初級農業生產而言，兩者均有高度對抗天災脆弱性或易損性 (vulnerability to disasters) 的共同環境限制，故為氣候正義 (climate justice) 重要的研究對象與議題。進言之，儘管原住民部落與平地農村社區的土地利用型態，均以農業生產為主，但兩者的基本差異在於原住民部落被鑲嵌於不同的空間與區位，特別是高山部落的生活空間範圍，經常涵蓋水源保護區、國家公園、自然保留區、野生動物保護區、野生動物棲息地及其他環境敏感地區，故對於氣候變遷的災害感受，遠較於平地農村與農民來得直接與嚴峻。Wang (2013) 在高山原住民部落因應氣候變遷之農業調適策略的質性研究結果，發現原住民部落對氣候變遷的農業衝擊感受，主要以農作物收成不佳與農業適耕地改變的情形最為嚴重。相對的，原住民部落在因應氣候變遷風險的農業調適措施，則著重於運用豐富的山林資源、在地生態知識及傳統價值體系來降低氣候變遷的農業風險危害，諸如改變農作物耕種方式、採用自然農法管理、選擇與遷種適耕地點，以減少農作物受到天災之威脅。此外，Sun & Yen (2012) 進一步指出石門水庫集水區的泰雅族原住民，因其傳統居住地區的交通偏遠、居住環境條件不利，在從事傳統初級產業時，必須融合土地倫理與傳統生態知識 (traditional ecological knowledge; TEK) 來尋求農業經營的永續發展，以避免氣候不正義現象。

儘管前述的研究成果，已初步呈現出高山農業發展與原住民部落的特殊社會生態體系，相較於平地農村與農業的發展，在因應氣候變遷相關災害時存在顯著的差異。但是，未來如何運用高山原住民部落所擁有的豐富的自然資本、多元的在地生態知識及獨特的傳統價值體系來研擬氣候變遷的農業調適策略，則缺乏客觀的實證數據來加以佐證。故本研究擬以問卷調查方式，以蒐集量化的數據來進一步分析氣候變遷災害對於原住民部落農業生產的影響，以及原住民部落如何運用地區資本來因應氣候異常現象對當地農業生產之負面衝擊。綜合上開研究背景的闡述，本研究目的嘗試釐清的研究問題包括：(1) 探討高山原住民部落對於氣候變遷災害嚴重性的感受；(2) 氣候異常現象對部落農業生產的影響程度；(3) 氣候變遷災害、地區資本項目與存量對於原住民部落農業生產的正負面影響結果。

氣候變遷風險之主觀評價

從社會風險管理的角度來看，強調人類社會如何因應氣候變遷風險威脅與降低氣候災害損失的議題，包括氣候變遷可能引發哪些災害風險？又哪些氣候災害風險會直接或間接影響到個別家戶的福祉？以及如何研擬適當的因應策略，以降低人類遭受氣候災害風險的危害程度，進而確保或增強弱勢族群的未來發展能力？ (Heltberg *et al*. 2008; Chen & Wang 2014) 事實上，氣候變遷影響農業的主要風險來源，在於增加極端天氣與天然災害等氣候異常現象的發生機率，而溫度與雨量的劇烈變化，對於原住民部落生活和高山農業生產造成極大的不確定性與威脅。因此，原住民部落農業可謂是氣候災害衝擊的高度敏感族群，有必要深入探討原住民對氣候變遷的災害風險認知與調適策略之間的關係，諸如原住民部落生活範圍內的季節溫度與雨量變化情形、天然災害發生頻率與強度的變化、農作物遭受氣候異常現象的危害損失程度，以及原住民部落所擁有的地區資本與因應氣候變遷風險之關聯性。

Su *et al*. (2013) 指出台灣 1999–2008 年期間，因天然災害導致農業損失總計約 850 億元，占十年農業總產值的 4.4%。此可能由氣候變遷衍生的天災損失，無論是溫度變化的夏季熱浪與冬季寒害，季節性降雨量變化的乾旱缺水與豪雨淹水，或是短期內侵襲的暴風雨、颱風、洪水、土石流等極端天氣事件，對於高度依賴自然資源的鄉村地區或農業部門，均將產生顯著且嚴峻的影響，特別是會進一步衝擊高山原住民部落的生活安全、生態環境、糧食安全及農業生產基礎。然而，由於原住民部落地處偏遠，且生活在氣象多變的高山地區，無論是氣候異常現象對於原住民日常生活的影響程度，或是對其部落農業生產的災損情形，均缺乏客觀精確的統計數據來加以呈現。故前人研究改以氣候變遷風險的認知與感受，主要調

查受訪者曾經歷或感受到的氣候變遷風險威脅的嚴重程度，以及氣候災害事件發生可能性，透過主觀評價的方式來掌握氣候變遷及其災害風險的潛在影響 (Chen & Wang 2014)。

從社會心理學的角度來看，氣候變遷對於人類社會的影響，不單單是探討人們直接與實質的受災經驗，而是關切其對氣候變遷及其引發災害的心理狀態與行為反應。因為在全球暖化的氣候變遷趨勢下，可能因為自然生態系統的變化，影響到其賴以維生的生計基礎，進而改變人與人之間的關係。以本研究地區氣候變遷及其災害風險的特殊性而言，氣候變遷風險對於尖石鄉泰雅族部落的主要威脅，係以極端氣候災害為主，尤其是高山聯外道路與農業生產的高度脆弱性。因為颱風與密集降雨所造成的崩塌、地滑與土石流等災情，進而阻斷研究地區的物資運送與產業經濟發展的命脈，為部落適居住與部落安全的最大隱憂。Wang (2013) 進一步指出，當部落農業受到風災、水災與氣候條件改變之影響，破壞部落原有的農業耕作條件與資源環境，已進一步衍生出部落青年人口加速外流的問題，而此離鄉決策行為背後，則潛藏著部落居民對於氣候變遷風險的認知成份與情感判斷。因此，有必要從風險認知的觀點，探討原住民對居住與生產環境遭遇氣候變遷的風險知覺與行為反應。

前人研究在分析原住民對於氣候變遷風險的認知時，特別強調環境風險的特性、風險來源與危害的瞭解程度、氣候風險的主觀評價及風險暴露的可能後果 (Chen & Wang 2014)。進言之，在氣候變遷風險的瞭解程度上，重點在於瞭解個人對氣候變遷資訊的關心程度，以及其所瞭解氣候變遷事實的正確性；氣候災後危害的主觀評價，則是個人對氣候災害風險情境的不確定性與可能發生的機率之判斷；至於個人暴露於氣候災害風險的可能後果，即聚焦在氣候災害風險可能造成的嚴重程度。由於本研究重點在於探究氣候變遷及其引發災害對於高山原住民生活和部落農業生產的影響程度，故在研究設計上，偏重於研究地區原住民對於氣候變遷災害，以及氣候異常現象對部落農業衝擊嚴重性之主觀評價。包括受訪者在過去 3 年內對於各項氣候災害危害嚴重程度的主觀感受，以及受訪者對於氣候異常現象所造成的部落農業生產損失之自評結果。

地區資本發展之氣候變遷因應策略

本研究對象為尖石鄉泰雅族部落，其社會生態體系 (social ecosystem) 的重要特徵，主要係位於石門水庫最上游集水區，屬於偏遠地區的高山原住民部落。具有高自然災害風險與高脆弱度 (Sun 2006)，並以 2004 年 8 月艾利風災對當地所造成環境災害最為嚴重，可視為尖石鄉後山部落在氣候災害與社會發展的關鍵事件 (Lin 2009)。其次，泰雅族部落生計對於自然資源的高度依賴性，不僅就業機會與收入來源受到限制，僅能從事農業或自然採集業活動，故其面臨自然災害的風險威脅，遠較於平地農村社區來得深遠和嚴峻。相對的，當地原住民部落因擁有豐富的自然與人文資產，特別是部落的傳統組織與 gaga 規範，則是泰雅族獨特擁有的社會文化資本，被視為泰雅族部落因應氣候變遷風險的重要地區資本 (Yen & Kuan 2004)。根據 Stückelberger (2009) 的實踐氣候正義指導方針，指出如何合理投入與分配有限的資源，為預防、減輕、適應氣候變遷災害風險的首要原則。其次，行動者在因應氣候變遷風險的心理狀態與行為調適上，應接受新的天氣條件，積極調整自身的生存情境與可能的選項，包括轉換新的生活方式、採用新的科技和生產模式、培養新的知識與因應能力。最後，應以整體地域發展思維，增加自然資源、財務資源、人力資源、社會資源、文化資源等資本總量，以減輕或適應氣候變遷的可能衝擊。

承前所述，由於原住民部落在地理區位上，與鄉村地區具備相似社會生態特徵，包括人口規模小、人口密度低、聚落稀疏且分散，並以農業經營或自然資源利用作為主要的社會經濟基礎。因此，原住民部落的社會發展亦適用於鄉村發展的理論與模式。就鄉村發展的理論內涵而言，歐盟在鄉村經濟發展行動連結－LEADER 計畫中，提出地域競爭力

(territorial competitiveness) 發展模式，並被 OECD (2006) 讚譽為鄉村治理的新典範。地區競爭力策略對於小型鄉村地區的認定方式，以是否擁有相同的實質/地理、經濟、社會、文化或政治特徵為指標。由當地居民自主建構成同質性單位，透過在地行動團體與地方發展計畫，強調如何將地區資本轉換成地域競爭力。在 2020 年歐盟凝聚政策規劃報告中，亦重申以地域發展模式來因應氣候變遷的風險與挑戰 (Böhme *et al*. 2011)。有鑑於此，本研究則從地域競爭力的觀點出發，強調居民參與、由下而上決策方式及地方資源的動員之外，更重視在地部落與外在世界的連動關係。尤其是原住民部落如何因應全球氣候變遷與國內社經結構重組的壓力，諸如自然天災的生存威脅、生態保育的環境意識、人口持續外流及農業因應氣候變遷風險等問題。

地區資本 (area's capital) 泛指地區內所有可構成地方資產的元素，包括有形與無形資本，或可從 8 項構成要素來區分之，分別為實質資源與管理、文化與地方認同、人力資源、知識技能與技術、地方制度與行政規章、活動與商業公司、市場與外部關係，以及地方意象與認知。若以地域競爭力作為因應氣候變遷風險的調適策略時，則建議由在地行動團體針對各類資本要素的評估結果，進一步篩選出可供地區競爭力計畫發展利用的資源要素與存量，諸如環境競爭力所需的資源項目為實質資源、人力資源、市場與外部關係，及地方意象與認知。社會競爭力，則著重於人力資源、文化與地方認同、知識技能與技術及地方制度與行政規章等要素。而經濟競爭力，則以實質資源、知識技能與技術、地方制度與行政規章、活動與企業，及市場與外部關係為主要的資本要素 (European Commission 2003)。此外，由於歐盟的地區資本之評估項目與分類方式，與我國國情與法制傳統略有差異，特別是我國自 2010 年推動農村再生政策後，已將農村地區 (含原住民部落) 的發展面向與內涵，予以明確的規範。亦即農村再生計畫，應包含農村社區整體環境改善、公共設施建設、個別宅院整建、社區產業活化、文化資產保存與活用、生態保育改善、農村社區公約、居民向心力與照顧服務及網路與資訊設施等。

本研究綜合前人研究，將地區資本重新歸納整併為環境資本、經濟資本、社會資本與知識資本 (Wang 2013; Lee *et al*. 2014)。整體而言，本研究所關注的尖石鄉泰雅族部落的社會生態體系，研究個案的自然環境資本，因位於石門水庫上游集水區，而與外部現代化社會的接觸與連結的相當晚；相對的，研究地區內的豐富自然資源與生物多樣性，也因為開發時間慢和開發程度低，而得以能完整保存原始的自然山林風貌與珍貴的神木群 (Su 2006)。其次，就研究地區的社會文化資本方面，主要由多個傳統、小型、獨立、特殊的泰雅族部落所構成的封閉型社會，故擁有獨特且濃厚的部落意識、世代承傳的部落文化傳統及高度的內部凝聚力等 (Yen & Kuan 2004)。相對的，研究地區的經濟資本發展現況困境，主要為地理的偏遠性與空間的孤立性，限制部落產業的發展機會，進而造成人口快速外流與人口嚴重老化等問題 (Sun 2006)。最後在知識資本上，則著重將部落世代承襲的傳統生態知識，應用在現代土地利用與自然資源管理方面，並發展出有別於科技法制化下的自然農業模式，近年來更透過將網路資訊系統來擴展知識的來源，並強化與外在世界的聯繫。

材料與方法

資料來源

本研究以新竹縣尖石鄉泰雅族部落作為研究對象，依照目前行政區劃來看，該地區包含馬美、李埔、宇老、玉峰、宇抬、下文光、石磊、平淪文、泰平、上抬耀、下抬耀、司馬庫斯、田埔、秀巒、錦路、養老、泰崗、新光、鎮西堡等 19 個原住民部落 (Sun & Yen 2012)。在尊重部落主體性與不打擾常規生活之研究調查倫理下，本研究隨機選取 9 個泰雅族部落，先行採面對面訪談部落意見領袖，並於 2013 年 2–12 月期間，委由部落長老或社區發展協會幹部協助發送與調查每一部落 15 份問卷，經剔除拒訪、漏答與填答異常的問卷

後，總計獲得有效樣本 114 份，有效問卷回收率 84.4%。

問卷設計

本研究旨在探討部落地區資本、氣候災害現象及原住民農業生產之關聯性。問卷調查內容之設計，主要延續「高山原住民部落因應氣候變遷之農業調適策略」的質性研究成果 (Wang 2013)，並參酌前人研究中有關農村地區因應氣候變遷風險量化問卷之題項設計 (Chen & Wang 2014; Lee *et al*. 2014)，將調查內容區分為受訪者基本資料、部落地區資本存量、氣候變遷災害、氣候異常對農業生產影響程度等 4 大部分，有關變數選取與變數定義的詳細內容，如表 1 所示。

社會人口變項：在樣本基本特徵的描述上，包含性別 (男性 = 1)、年齡、教育程度以國小以下、國中、高中職、大專以上學歷等 4 類虛擬變數表示 (是 = 1)、平均月收入則考量到樣本分布比例，以 2 萬元作為劃分標準 (2 萬元以下 = 1)。

地區資本存量：有關部落地區資本存量主要參考歐盟調查有形與無形地方資源的構成要素 (European Commission 2003)，並依據我國農村社區資源盤點的重點 (Wang 2013; Lee *et al*. 2014)，將其分為 4 大構面與 8 個題項來加以測量，調查受訪者在過去 3 年內 (2010–2012 年期間) 對於部落生活地區內各項資源的主觀評價，包括環境資本 (含聚落自然環境與公共建設、生態保育情形)、經濟資本 (含部落產業發展、年輕人返鄉就業機會)、知識資本 (含傳統文化與技術承傳、網路系統與資訊取得)、社會資本 (含部落居民向心力、互助組織與價值體系)。各題項採用四點李克特尺度 (非常豐富 = 4、豐富 = 3、貧乏 = 2、非常貧乏 = 1)，並以加總分數來代表該面向的資本存量。業經內部一致性的信度檢驗，Cronbach's α 為 0.82，顯示此量表具有良好的信度。

氣候變遷災害：有關氣候變遷災害的嚴重程度，主要調查受訪者在過去 3 年內對於部落生活地區內曾經遭遇各項氣候災害危害嚴重程度的主觀評價，包括複合型災害 (如颱風同時帶來強風、豪雨、洪水和土石流)、降雨量異常、溫度劇烈升降所造成極端天氣災害等 3 個題項，各題項採用 4 點李克特尺度 (非常嚴重 = 4、嚴重 = 3、不嚴重 = 2、從未發生過 =

表 1. 受訪者樣本基本特徵與描述性統計 (N = 114)。

Table 1. Sample characteristics and descriptive statistics of regression variables (N = 114).

Characteristics	Mean/Percentage	SD[z]
Socio-demographic characteristics		
Gender (Male = 1)	0.50	
Age	50.75	12.84
Elementary or lower	22.81	
Junior high school	34.21	
Senior high school	25.44	
College or higher	17.54	
Monthly income under NT$ 20,000	67.55	
Climate disaster severity	6.08	1.92
Area capital profile		
Environmental capital	4.65	1.28
Economic capital	4.30	1.55
Knoweldge capital	4.52	1.51
Social capital	4.65	1.42
Impact of climate disorder on agricultural production	8.24	2.20

[z] SD: standard deviation.

1)，並以加總分數來代表受訪者對氣候變遷災害的整體感受。業經內部一致性的信度檢驗，Cronbach's α 為 0.91，顯示氣候變遷災害測量具有良好的信度。

氣候異常對農業生產的影響程度：由於研究地區泰雅族部落係以農業生產作為主要經濟活動，故氣候異常現象對於農業經營的生產條件、產量與品質，均會造成明顯的衝擊，特別是極端天氣事件的增加、降雨頻率和強度的加劇、溫度升降的劇烈變化，皆可能對部落農業生產產生潛在或立即的威脅。因此，在測量氣候異常對農業生產的影響上，分別調查受訪者對氣候異常現象所造成的農作物產量減少、農作物品質降低、原有生長條件受破壞，及部落農業生產的整體損失之嚴重程度 (非常嚴重 = 4、嚴重 = 3、不嚴重 = 2、從未發生過 = 1)，並以 4 題加總分數來代表受訪者對於氣候異常衝擊農業生產的嚴重程度，此測量題項的內部一致性的信度為 0.86。

資料分析方法

本研究以統計軟體 SPSS19.0 進行資料登錄與統計分析，首先以描述性統計分析受訪者的性別、年齡、教育程度、平均月收入等社會人口變項的基本特徵，以及本研究關鍵變數的平均數與標準差，包含氣候異常對農業生產的影響 (即依變數)、地區資本存量與氣候變遷災害 (即解釋變數)。其次，本研究為探討氣候變遷災害、地區資本與原住民部落農業生產之關聯性，則採用最小平方法的多元迴歸模型來分析部落地區資本存量、氣候變遷災害對於部落農業生產的影響效果。

結果與討論

受訪者樣本基本特徵

在社會人口變項方面，114 位受訪者的男女性別各占一半，比例相當；平均年齡為 50.75 歲，教育程度則以國中畢業者比例最高 (34.21%)，高中職比例次之 (25.44%)，大專以上學歷者之比例最少 (17.54%)，顯示研究部落居民教育程度偏低，學歷在國中以下的比例超過 57%。在平均月收入上，以每月收入 2 萬元以下為受訪者居多 (占 67.55%)、2–4 萬元次之 (18.42%)、4–6 萬元位居第 3 (7.93%)，6 萬元以上的比例為 6.10% (如表 1 所示)。

從表 1 中可看出本研究所關注的「氣候異常對農業生產的衝擊」上，受訪者自評氣候異常現象對當地部落農業生產的影響程度平均為 8.24 分，其中以農作物品質降低最為嚴重 (2.08 ± 0.63)、農作物原有生長條件遭受破壞次之 (2.07 ± 0.58)、第三為部落農業整體損失 (2.05 ± 0.64)、農作物產量減少居末 (2.04 ± 0.62)。可見近 3 年來氣候異常現象對於研究部落地區農業生產的衝擊，雖然有負面的影響，但嚴重程度僅屬輕微。另外，就關鍵的解釋變數而言，受訪者對於過去 3 年內氣候變遷災害嚴重程度的主觀評價平均為 6.08 分，其中以降雨量異常最為嚴重 (2.21 ± 0.69)、複合型災害 (2.11 ± 0.78) 次之，溫度劇烈升降的災害風險 (1.80 ± 0.73) 居末，顯見降雨量異常、颱風、豪雨、洪水和土石流的複合型災害為影響原住民部落最為嚴重的氣候變遷災害。相對的，被視為有助於因應氣候變遷災害的地區資本存量而言，4 大構面資本存量的自評結果，環境資本與社會資本平均分數均為 4.65，並列第 1，其他依序為知識資本 (4.52 ± 1.51)、經濟資本 (4.30 ± 1.55)。此結果符合本研究個案地區係由原始山林與泰雅族部落共同建構出特殊的社會生態體系，故擁有豐富的自然環境資本與獨特的社會文化資本，此泰雅族部落的傳統生活與大自然的和諧共存方式，亦將是原住民部落農業因應氣候變遷衝擊的最重要的地區資本 (Wang 2013)。

就部落地區資本的細項測量題項來看，前 3 名分別為生態保育情形 (2.49 ± 0.79)、部落居民向心力 (2.39 ± 0.84)、傳統文化與技術承傳 (2.31 ± 0.84)，並以年輕人返鄉就業機會的自評分數最低 (2.17 ± 0.88)。此分析結果可發現研究個案地處於偏遠的高山地區，地理的偏遠性與空間的孤立性，導致高山原住民部落生活與生計活動，必須高度依賴山林資源與自然生態系統，故會形成緊密社會連帶關係、相互扶持的文化規範與互助組織，進而形塑出濃厚的部落意識與向心力，並發展出在地的生態知

識、耕種技術及生態保育實務；相對的，也因為研究地區的工作機會與收入來源受限於農業與自然採集等經濟活動，故在有限的經濟資本下，不利於部落青年返鄉就業。

氣候異常影響農業生產之迴歸分析

本研究使用多元迴歸模式分析氣候異常影響農業生產之相關因素及其影響效果。就整體迴歸模型的配適度而言，迴歸模型可解釋變異量比為 43% (R^2)，校正後的模型解釋力為 36% (adjusted R^2)。而在迴歸模式配適度的檢定上 (F 值為 6.10)，顯示依變數和所有自變數之間具有統計顯著性 ($P < 0.001$)，亦即棄卻所有自變項影響效果同時為零的虛無假設。從個別自變項對於「氣候異常影響農業生產嚴重性」的分析結果，在社會人口變數的影響效果方面，顯示受訪者的年齡愈高 (β = 0.18)、高中教育程度者 (對照於國小以下學歷者，β = 0.28)、平均月收入低於 2 萬元者 (對照於高於 2 萬元者，β = 0.04)，其認為氣候異常現象對農業生產有顯著的負面影響，亦即自評的嚴重性愈高，如表 2 所示。上開的分析結果，符合前人研究的發現 (Chen & Wang 2014)，亦即氣候異常現象對於以務農為生的老年人、中等教育程度、收入較低群體的影響，其嚴重程度的主觀感受也較高。

從表 2 的迴歸分析結果中，可看出受訪者感受到的氣候變遷災害程度，對於「氣候異常影響農業生產的嚴重性」有正向的影響效應 (β = 0.61)，且其影響效果高於所有的解釋變數。此分析結果符合本研究的預期假設，亦即高山原住民部落的農業經營，在面臨氣候變遷的天候情境改變，特別是氣候變遷災害愈高時，其農業生產的質量、耕種環境與農業產值，其對抗天災的脆弱性 (vulnerability) 也較高，亦即愈容易造成較高的農業生產損失。其次，從部落地區資本存量的影響來看，迴歸分析結果顯示：環境資本、知識資本、社會資本對於受訪者自評的「氣候異常影響農業生產之嚴重性」，有顯著的負向影響效應 ($P < 0.01$)，其標準化的迴歸係數 β，有低到高分別為 -0.01、-0.08、-0.17，並以社會資本的影響效果最高；

表 2. 氣候異常影響農業生產之多元迴歸分析結果 (N = 114)。

Table 2. Estimation of Multiple Regression Equation. (Dependent variable: Impact of climate disorder on agricultural production, N = 114)

	Unstandardized coefficient		Standardized coefficient
Explanatory variable	B	SE	Beta
Climate disaster severity	0.70	0.10	0.61***
Area capital Profile			
Environmental capital	-0.01*	0.27	-0.01*
Economic capital	0.31	0.23	0.22
Knoweldge capital	-0.11*	0.24	-0.08*
Social capital	-0.26**	0.19	-0.17**
Socio-demographic characteristics			
Gender (ref. Female)	0.24	0.39	0.05
Age	0.03**	0.02	0.18**
Education (ref. Elementary or lower)			
Junior high school	0.08	0.58	0.02
Senior high school	1.37**	0.60	0.28**
College or higher	0.96	0.63	0.17
Monthly income under NT$ 20,000	0.16*	0.41	0.04*
Constant	0.73	1.69	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; $R^2 = 0.43$, adjusted $R^2 = 0.36$, F-value = 6.10 ($P < 0.001$).

相對的，經濟資本則無顯著的影響效果。

進言之，從地區競爭力發展模式的觀點來看，原住民部落所擁有的地區資本項目與存量，為因應氣候變遷及其引發災害風險的重要手段 (European Commission 2003; Wang 2013)，故本研究推論不同面向的地區資本存量愈高者，其自評氣候異常現象對農業生產的負面影響程度愈低。因此，表 2 迴歸分析結果符合本研究所預期的假設，亦即部落居民向心力愈強、互助組織與價值體系愈多 (即社會資本存量愈高)，愈可以降低氣候異常現象對部落農業生產的負面影響。相似的，聚落自然環境、公共建設與生態保育成果愈完善 (即自然資源存量愈高)、傳統文化與技術承傳愈多、網路系統與資訊取得愈便利 (即知識資本存量愈高)，均有助於減少氣候異常現象影響部落農業生產的嚴重程度；相對的，經濟資本存量的高低 (即部落產業發展成效、年輕人返鄉就業機會多寡) 則無顯著的影響效應。綜合而言，本研究發現：以發展地區資本作為原住民部落農業面對氣候變遷風險的因應策略時，應著重在強化原住民部落的社會資本、知識資本與環境資本的存量，以及其資本運用方式。亦即有關單位應鼓勵與協助高山原住民永續利用豐富的山林資源，維持泰雅族部落的傳統生活方式與部落社會網絡，加強在地生態知識的承傳與累積，以降低氣候變遷及其引發天然災害對部落農業經營的負面衝擊；相對的，在經濟面向的地區資本上，高山地區的原住民部落，則因地理偏遠性、空間孤立性、自然資源的高依賴性，進而造成原住民部落的社經條件不利性及對抗天災風險的脆弱性。故以提高地區經濟資本的因應策略，對於降低「氣候異常影響部落農業生產的嚴重性」上，其成效相當有限。

本研究從原住民部落社會發展的面向，探討尖石鄉泰雅族農業發展遭受氣候變遷引發的氣候異常現象與氣候災害事件的影響程度，以及泰雅族部落運用地區資本因應氣候變遷風險的可能性與限制。本研究結果可作為高山原住民部落農業發展，研擬氣候變遷調適策略的規劃參考。重要的研究發現，包括：(1) 環境資本與社會資本為研究個案部落自評最為豐富的地區資本；(2) 在迴歸分析結果中，則確認受訪者感受到的氣候災害程度愈為嚴重時，也會提高其對「氣候異常影響農業生產的嚴重性」的主觀評價；(3) 部落地區資本存量愈高，將會顯著降低與減緩「氣候異常影響農業生產的嚴重性」，然而不同面向地區資本的影響效果不一，其中以部落社會資本因應「氣候異常影響農業生產」的調適效果最高；相對的，經濟資本則無顯著的影響效果。

綜合上開研究結果，本研究引藉歐盟的地域競爭力發展模式，分析原住民部落運用地區資本以減緩氣候變遷風險衝擊農業生產的因應策略時，重要的政策建議，首先應尊重與厚實原住民部落的社會資本，維持泰雅族部落的傳統生活方式與部落社會網絡。因為凝聚強烈的部落意識及高度的內部向心力，落實部落互助組織與共享價值，此減緩部落農業遭受氣候異常現象負面衝擊的首要調適策略，有助於部落居民進一步自主發展出農業共同產銷、合作儲水灌溉、平時作物保護、災前搶收與災後復耕等部落農業防減災措施。其次，保育與強化部落的環境資本，透過豐富的山林資源，可以激發出傳統的農業生態知識、耕種技術及生態保育實務，使原住民部落農業可以因應氣候變遷及其災害的衝擊，如鼓勵原住民部落在氣候條件改變下，發展與大自然和諧共存的在地自然農法，以兼顧部落農業生產與保護環境生態的永續治理。最後，在知識資本的氣候變遷因應策略上，有關單位則應加強與建置部落傳統生態知識與傳統技術，集結部落內部知識資本之力量，進行傳統農耕文化與生產技術承傳，並透過網路及資訊設施，累積與傳佈部落農業經營因應氣候災害衝擊的防減災操作實務與相關作業流程，如採取栽種多樣化作物、分散作物栽種地，以防範農作物損失的災害風險。

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引用文獻

Böhme, K., P. Doucet, T. Komornicki, J. Zaucha, and D. Świątek. 2011. How to Strengthen the Territorial Dimension of Europe 2020 and the EU Cohesion Policy. Polish Presidency of the Council of EU. Warsaw, Poland. 97 pp.

Chen, M. F. and J. H. Wang. 2014. Risk perception and adaptation of agricultural extension agents on climate change. J. Farmer Organ. 19:55–100. (in Chinese with English abstract)

European Commission. 2003. Fact sheet: Rural development in the European Union. DG Agric. Rural Devel., Brussels. http://ec.europa.eu/agriculture/publi/fact/rurdev2003/en.pdf (visit on 6/18/2015)

Heltberg, R., S. L. Jorgensen, and P. B. Siegel. 2008. Climate Change, Human Vulnerability, and Social Risk Management. Social Development Department, World Bank.Washington, DC. 56 pp.

Lee, K. Y., L. C. Lan, C. L. Fang, and J. H. Wang. 2014. Impact of participation in Rural Regeneration Program on community development satisfaction of rural residents. Surv. Res. Method Appl. 32:11–51. (in Chinese with English abstract)

Lin, Y. R. 2011. Politicizing nature: The Maqaw National Park controversy in Taiwan. Capital Nat. Soc. 22:88–103.

Organization for Economic Cooperation and Development (OECD). 2006. The New Rural Paradigm: Policies and Governance. OECD Pub. Paris. 168 pp.

Stückelberger, C. 2009. Why and for whom should we care? Environmental ethics, responsibility and climate. Fudan J. 2009:68–79.

Sun, C. T. 2006. Analysis of Indigenous Conservation and Utilization on CPR: Two CPR Self Governing Cases of Atayal Tribe in Taiwan Indigenes. Master thesis, Department of Land Economics, National Chengchi University. Taipei, Taiwan. 158 pp. (in Chinese)

Sun, C. T. and A. C. Yen. 2012. An investigation of watershed management and land ethics- A case of indigenous communities on upstream Shimen reservoir catchments area. J. Geog. Sci. 66:21–51. (in Chinese with English abstract)

Su, P. J. 2006. The Role of Aboriginal Communities in Natural Resources Management: Cases on Atayal Smangus, Cinsbu, and Smagus Tribes in Hsinchu County. Master Thesis, Department of Political Science, National Chung Cheng University. Chiayi, Taiwan. 153 pp. (in Chinese with English abstract)

Su, Y. J., M. L. Cheng, and J. H. Wang. 2013. Spatial analysis of crop losses caused by natural disasters. Taiwanese Agric. Econ. Rev. 18(2):1–47. (in Chinese with English abstract)

United Nations Framework Convention on Climate Change (UNFCCC). 2009. Report of the Conference of the Parties on Its Fifteenth Session. December 7–19, 2009. Copenhagen, Denmark. 43 pp.

Wang, J. H. 2013. Agricultural adaptation to climate change among highland indigenous tribes. J. Agric. Assoc. 14:491–505. (in Chinese with English abstract)

Yen, A. C. and D. W. Kuan. 2004. Traditional institution and the institutional choice: Two CPR self governing cases of Atayal tribe in Taiwan indigenes. J. Geogr. Sci. 37:27–49.

Association among Climate Disaster, Area Capital and Agricultural Production of Indigenous Peoples

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Abstract

Wang, J. H. 2016. Association among climate disaster, area capital and agricultural production of indigenous peoples. J. Taiwan Agric. Res. 65(3):286–295.

Although there is an increasing body of literature indicates that climate change and its caused extreme weather events have seriously threatened lifestyle of indigenous tribe and mountain agricultural production. However, relatively little is known about the quantitative association among climate disaster, area capital and agricultural production of indigenous peoples. Paying special attention to territorial competitiveness of ingidenous area, the objective of this study is to examine the extent to which climate disorder and disaster, area capital profile and socio-demographic factors are associated with indigenous agricultural production. Data used were drawn from the “Tayal tribe survey in Jianshi Township” in 2013, including 114 valid samples. This study employed the ordinary least squares (OLS) regression model to estimate the effects of indigenous area capital and climate disaster severity on their agricultural production. The results show that crop quality decrease and deterioration of environment for crop production are the worst consequences resulted from climate disorder. As expected, different dimensional area capitals have positive contribution to reduce the impact of climate disorder on indigenous agricultural production significantly, except the economic capital. Several policy implications for agricultural adaptation to climate change for indigenous agricultural development can be inferred from these findings. The first prority of adaptative stretagy is to strengthen social networks of tribal solidarity among indigenous peoples. The social dimension of area capital can contribute to enhance climate and disaster resilience in the context of indigenous agricultural development. For example, to maintain traditional way of life and social networks of Tayal tribes can contribute to reducing climate change risk for agriculture through utilizing indigenous mutual supporting organization and sharing values system.

Key words: Indigenous tribe, Climate disaster, Area capital, Territorial competitiveness.

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成蟲飼料添加新尼古丁類與抗生素類殺蟲劑對瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] (Diptera: Tephritidae) 之毒效測試

賴信順 [1,*]

摘要

賴信順。2016。成蟲飼料添加新尼古丁類與抗生素類殺蟲劑對瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] (Diptera: Tephritidae) 之毒效測試。台灣農業研究 65(3):296–305。

瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 為葫蘆科作物重要害蟲，誘殺性餌劑為目前政府推薦的主要防治方法。本研究在室內以非選擇性 (僅提供含毒食餌) 與選擇性 (同時提供含毒與無毒食餌) 試驗，篩選對瓜實蠅具良好毒效的新尼古丁與抗生素類殺蟲劑，作為餌劑添加藥劑的參考，期進一步減少對環境與農民健康的傷害，同時增加用藥選擇的多樣性，減緩害蟲抗藥性的產生。非選擇性試驗結果顯示，新尼古丁類殺蟲劑賽速安、可尼丁和達特南，以及抗生素殺蟲劑因滅汀、賜諾特和賜諾殺，添加於成蟲食餌中對瓜實蠅具有良好的毒殺效果。上述抗生素類殺蟲劑，對瓜實蠅 24 h 致死率約 60%，高於新尼古丁類殺蟲劑所造成約 50% 的死亡率，但累積 72 h 後 2 類殺蟲劑對瓜實蠅的致死率皆高達 95%。試驗亦顯示，因滅汀和賜諾特對瓜實蠅的毒殺效率，相較於目前政府推薦的東方果實蠅防治用餌劑之添加藥劑賜諾殺為快，而賽速安、可尼丁和達特南的毒殺效率也與賜諾殺相當。選擇性試驗顯示，此 2 類殺蟲劑不會對瓜實蠅造成明顯忌避作用，同時也證實上述藥劑對瓜實蠅具有確實的毒殺效果。綜合而言，本研究顯示這些篩選出的殺蟲劑以及效果稍微次之的阿巴汀，皆極具進一步探討其添加於優良餌劑中防治瓜實蠅的潛力。

關鍵詞：瓜實蠅、新尼古丁類殺蟲劑、抗生素類殺蟲劑、餌劑。

前言

瓜實蠅 [*Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)] 是葫蘆科作物的重要害蟲，幼蟲蛀食所造成的危害，會使果實失去商品價值而導致農民嚴重的經濟損失。瓜實蠅成蟲將卵產在植物組織內，孵化後幼蟲直接蛀食果實、啃食花器或莖部，發育至老熟幼蟲時跳離植株藏匿於土中化蛹。因此，瓜實蠅在卵期、幼蟲期與蛹期皆不容易防治，而成蟲期就成為瓜實蠅防治的關鍵時期。針對其生態習性與行為模式，整合清園、套袋、誘殺與噴施農藥等完整防治策略，才能降低瓜實蠅對作物的危害 (Dhillon *et al*. 2005)。在上述防治技術中，噴施農藥是最被廣泛運用的技術，但將農藥直接噴施作物上會增加農藥殘留過量的風險，並且對環境與生態造成負面影響。因此，在副費洛蒙 (parapheromone) 或蛋白質餌劑內添加農藥，對果實蠅類成蟲進行誘殺，除了降低族群密度外，亦能降低施用農藥所造成直接與間接的影響 (Ansari *et al*. 2012)。

目前國內推薦瓜實蠅的誘殺方式，有懸掛含有機磷類殺蟲劑的 85% 克蠅香混合溶液，以及於園區周圍點噴混合 25% 馬拉松可濕性粉劑的蛋白質水解物，或點噴稀釋的 0.02% 賜

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諾殺濃餌劑等 (Fei *et al*. 2010)。有機磷類殺蟲劑具廣效性的殺蟲能力，但國內報告指出果實蠅類對有機磷類殺蟲劑已有抗藥性 (Hsu & Feng 2002; Hsu *et al*. 2004)，而在餌劑內添加有機磷類殺蟲劑，可能會提升該果實蠅類族群對有機磷類殺蟲劑的抗藥性 (Hsu *et al*. 2015)。相較於高毒性的有機磷類殺蟲劑，新尼古丁類與抗生素類殺蟲劑對哺乳類生物的毒性較低且對防治害蟲的種類具選擇性 (Ghanim & Ishaaya 2011)。新尼古丁類殺蟲劑的主要作用機制，是與尼古丁乙醯膽鹼受體 (nAChRs) 結合，干擾乙醯膽鹼神經訊息之傳遞，造成昆蟲過度興奮和癱瘓而死亡，該類農藥有益達胺、賽果培、賽速安、亞滅培、可尼丁與達特南等 (Tomizawa & Casida 2005)。抗生素類殺蟲劑，主要殺蟲機制屬於神經性毒效，該類農藥純化自不同放線菌的次級產物，包括由 *Streptomyces avermitilis* 發酵產物純化的阿巴汀與其半合成衍生物純化的因滅汀，由 *S. hygroscopicus* 發酵產物純化的密滅汀以及由 *Saccharopolyspora spinosa* 天然代謝物純化的賜諾殺與賜諾特 (Lasota & Dybas 1991; Chio 2011)。

為了篩選出能夠取代有機磷類殺蟲劑添加於瓜實蠅餌劑內的低毒性藥劑，本研究挑選市售 6 種新尼古丁類殺蟲劑與 5 種抗生素類殺蟲劑，分別與液體成蟲飼料混合成 5 種濃度的含毒食餌，藉由非選擇性試驗 (僅提供含毒食餌供瓜實蠅取食) 與選擇性試驗 (同時提供含毒與無毒食餌供瓜實蠅選擇)，篩選出對瓜實蠅不具忌避作用且具優良毒效的藥劑，以及其添加於液體成蟲飼料內的有效濃度，並且與推薦的賜諾殺濃餌劑相比較，以冀未來作為瓜實蠅整合性管理技術應用的參考。

材料與方法

供試昆蟲

將受瓜實蠅為害的胡瓜 (*Cucumis sativus* L.)，置於溫室內的白色飼養帳 (60 cm × 60 cm × 60 cm)，伺瓜實蠅羽化之後，以 2 級砂糖和酵母水解物 (yeast hydrolysate enzymatic, MP Biomedical, Irvine, CA) (4 : 1) 及沾水棉花供瓜實蠅成蟲取食。為模擬瓜實蠅族群在田間的繁衍情形，因此輪流以南瓜 (*Cucurbita moschata*)、絲瓜 (*Luffa cylindrical*) 與胡瓜供其在飼養帳內產卵和取食，建立長期的供試蟲源。

試驗藥劑配製

食餌：將 2 級砂糖、酵母水解物與水以 4 : 1 : 5 (w/w/w) 的比例混合製備成液體成蟲飼料 (Hsu *et al*. 2004)，作為食物餌劑，以棉片 (3.5 cm × 3.5 cm × 0.8 cm) 吸收 12 mL 的食餌作為食餌棉片。

供試藥劑：試驗用殺蟲劑皆為成品農藥，包括 20% 亞滅培水溶性粉劑 (acetamiprid，台灣住友商事股份有限公司)、16% 可尼丁水溶性粒劑 (clothianidin，立農化學股份有限公司)、25% 賽速安水溶性粒劑 (thiamethoxam，台灣先正達股份有限公司)、20% 達特南水溶性粒劑 (dinotefuran，惠光股份有限公司)、18.2% 益達胺水懸劑 (imidacloprid，台灣科麥農有限公司)、40.4% 賽果培水懸劑 (thiacloprid，台灣拜耳股份有限公司)、2% 阿巴汀乳劑 (abamectin，台灣先正達股份有限公司)、2.15% 因滅汀乳劑 (emamectin benzoate，台灣先正達股份有限公司)、1% 密滅汀乳劑 (milbemectin，大勝化學工業股份有限公司)、5.87% 賜諾特水懸劑 (spinetoram，台灣道禮股份有限公司)、2.5% 賜諾殺水懸劑 (spinosad，台灣道禮股份有限公司) 與 0.02% 賜諾殺濃餌劑 (spinosad，台灣道禮股份有限公司)。

含毒食餌配置方法：模擬田間以食物餌劑誘殺瓜實蠅，藥劑調配比照植物保護手冊中所推薦的方式 (Fei *et al*. 2010)。0.02% 賜諾殺濃餌劑分別以 1、2、4 與 8 倍的水稀釋，於均勻混合後，4 種溶液與原液各吸取 12 mL，以棉片吸收作為供試含毒棉片。其餘供試農藥則是依蛋白質水解物與殺蟲劑的重量比配置成含毒食餌，於 200 mL 的液體飼料中分別加入 2、1、0.4、0.2 及 0.1 g 的供試藥劑，成為稀釋 100、200、500、1,000 及 2,000× 的溶液。為使農藥與液體飼料能均勻混合，因此以震盪培

養器混合 24 h 後，吸取 12 mL 的溶液，以棉片吸收作為供試含毒食餌棉片。

毒效試驗

試驗以餵食方式進行非選擇性與選擇性試驗，為模擬瓜實蠅族群在田間的情形，因此採用混合蟲源以篩選對瓜實蠅族群有較佳毒性的殺蟲劑。試驗前 72 h 於供試蟲源飼養帳內逢機取出 60–70 隻瓜實蠅成蟲移至方型飼養籠 (30 cm × 30 cm × 30 cm) 內，以食餌棉片進行餵食，於試驗開始前清出自然死亡的成蟲，實際試驗的瓜實蠅數至少 50 隻。

非選擇性試驗：不同藥劑與濃度個別測試，處理組僅提供含毒食餌棉片供瓜實蠅取食。處理組籠內分別放入不同濃度的供試含毒食餌棉片，對照組籠內僅放無毒食餌棉片，試驗採逢機完全區集設計 (RCBD)，每處理與對照組 3 重複，試驗開始後第 24、48 與 72 h 調查並紀錄籠內死亡蟲數與存活蟲數。

選擇性試驗：處理組同時提供含毒與無毒食餌棉片供瓜實蠅進行取食選擇。挑選前項試驗中經 72 h 後對瓜實蠅之平均累積死亡率大於 80% 的農藥與濃度進行選擇性試驗，不同藥劑與濃度個別測試。試驗時處理組籠內分別放入供試含毒食餌棉片與無毒食餌棉片各一，對照組籠內則僅放無毒食餌棉片，試驗採逢機完全區集設計 (RCBD)，每處理與對照組 3 重複，試驗開始後第 24、48 與 72 h 調查並紀錄籠內死亡蟲數與存活蟲數。

資料分析

各組數據先經角度轉換 (angular transformation, arcsin $\sqrt{P}$，P 為原始資料)，再利用 SAS/EG 套裝統計分析軟體進行變方分析 (analysis of variance; ANOVA)，達顯著性差異者再進行 Fisher 最小顯著差異性測驗 (Fisher's least significance difference test; LSD test)，以 5% 水準檢測處理間的差異顯著性。

結果

非選擇性試驗

新尼古丁類殺蟲劑的試驗結果顯示，含可尼丁、達特南或賽速安的食餌對瓜實蠅具有最強的毒殺效果，含濃度 2,000× 賽速安、2,000× 可尼丁及 1,000× 達特南的食餌於 24 h 後可造成約 40% 的瓜實蠅死亡率，而將賽速安濃度提高到 500×、可尼丁提高到 1,000×，瓜實蠅 24 h 後的死亡率可提升至 50%；上述毒餌對瓜實蠅於 48 h 後的累積死亡率相較於 24 h 可再提升約 30%；72 h 後瓜實蠅累積死亡率於含濃度 2,000× 賽速安、1,000× 可尼丁及 1,000× 達特南的食餌皆達約 95%。含濃度 2,000× 以上亞滅培的食餌，對瓜食蠅 72 h 的累積死亡率雖達 85% 以上，但 24 h 的死亡率僅約 20%。含益達胺和賽果培的食餌則對瓜實蠅毒殺效果不佳，各濃度於 24 h 後的死亡率多低於 10%，48 h 後的死亡率也多在 30% 以下 (表 1)。抗生素殺蟲劑方面，含濃度 2,000× 因滅汀、2,000× 賜諾特、200× 賜諾殺的食餌以及 8× 賜諾殺濃餌劑，24 h 後瓜實蠅死亡率約達 60%，48 h 後的死亡率超過 80%，72 h 後上述 4 種毒餌除了賜諾殺濃餌劑 (83%) 外，其餘死亡率皆高達約 95%。未經稀釋的賜諾殺濃餌劑對瓜實蠅的毒殺效果不佳，雖然 72 h 的累積死亡率可達 70%，但 24 h 的死亡率低於 10%；受測之含阿巴汀食餌對瓜實蠅 24 h 死亡率僅約 30%，但濃度 1,000× 以上的食餌 72 h 之累積死亡率皆達約 85%；而含密滅汀的食餌 72 h 後所造成的瓜實蠅累積死亡率仍低於 40% (表 2)。

選擇性試驗

將以上經非選擇性試驗篩選出之對瓜實蠅具有良好毒效的食餌，進一步以選擇性試驗測試其對瓜實蠅的忌避作用。結果顯示，在同時提供瓜實蠅含新尼古丁殺蟲劑食餌以及無毒食餌的情況下，含濃度 2,000× 賽速安、可尼丁和達特南的食餌 24 h 所造成的死亡率皆低於 30%，48 h 約 50%，72 h 則不高於 75%，死亡率隨時間增長而增加。含亞滅培的食餌相較下對瓜實蠅的毒效較低，但所造成的死亡率亦隨時間增長而增加 (表 3)。含因滅汀、賜諾特、賜諾殺和阿巴汀等抗生素殺蟲劑的食餌，在選擇性試驗中各時段所累積的瓜實蠅死亡率，皆較非選擇性試驗稍低。而賜諾殺濃餌劑之死亡

表 1. 新尼古丁殺蟲劑與食餌以不同比例混合於非選擇性試驗中對瓜實蠅之平均死亡率。

Table 1. Mean mortalities of *Bactrocera cucurbitae* exposed to food baits incorporated with different dilution rates of neonicotinoids in no-choice tests.

Insecticide	Dilution rate (×)	% Cumulative mortality (Mean ± SD)[z]		
		24 h[y]	48 h	72 h
Acetamiprid 20% SP	100	26.0 ± 7.0 a	62.8 ± 3.8 a	95.8 ± 1.8 a
	200	24.4 ± 4.6 a	48.2 ± 6.6 a	95.3 ± 1.6 ab
	500	24.7 ± 7.2 a	59.1 ± 9.5 a	90.4 ± 2.9 bc
	1,000	19.3 ± 3.7 a	47.4 ± 6.2 a	85.4 ± 4.3 c
	2,000	17.5 ± 4.5 a	47.7 ± 7.0 a	84.5 ± 4.7 c
CK		3.1 ± 3.0 b	4.6 ± 4.1 b	6.2 ± 4.1 d
Clothianidin 16% SG	100	53.2 ± 2.1 a	80.3 ± 2.2 a	96.8 ± 1.6 ab
	200	48.4 ± 0.8 a	78.1 ± 1.7 a	97.9 ± 0.9 a
	500	52.6 ± 2.8 a	80.9 ± 4.1 a	95.2 ± 3.2 ab
	1,000	47.1 ± 0.5 a	77.0 ± 2.0 a	93.2 ± 2.4 b
	2,000	39.1 ± 9.1 b	55.0 ± 3.4 b	80.8 ± 5.2 c
CK		0.0 ± 0.0 c	2.1 ± 0.9 c	2.1 ± 0.9 d
Dinotefuran 20% SG	100	44.7 ± 5.4 ab	79.2 ± 2.8 a	96.9 ± 1.6 a
	200	46.0 ± 1.9 a	77.0 ± 3.4 a	96.0 ± 3.2 a
	500	39.8 ± 3.6 bc	77.6 ± 4.3 a	93.2 ± 5.0 a
	1,000	37.6 ± 3.3 c	72.2 ± 7.4 a	93.3 ± 2.5 a
	2,000	30.2 ± 2.1 d	57.4 ± 0.5 b	80.5 ± 0.7 b
CK		0.0 ± 0.0 e	2.1 ± 0.9 c	2.1 ± 0.9 c
Imidacloprid 18.2% SC	100	13.1 ± 5.2 a	35.1 ± 15.5 a	65.3 ± 5.8 a
	200	7.0 ± 0.9 ab	27.6 ± 7.4 ab	54.6 ± 6.5 ab
	500	3.7 ± 1.0 bc	22.5 ± 9.9 ab	44.7 ± 6.0 bc
	1,000	5.9 ± 4.1 b	21.5 ± 14.6 ab	36.4 ± 14.7 c
	2,000	2.7 ± 0.9 bc	13.4 ± 1.9 b	32.3 ± 10.6 c
CK		1.6 ± 1.6 c	1.6 ± 1.6 c	3.2 ± 3.2 d
Thiacloprid 40.4% SC	100	10.2 ± 2.6 a	28.0 ± 3.5 a	70.9 ± 3.5 a
	200	9.3 ± 5.1 a	24.0 ± 9.0 a	60.6 ± 2.4 b
	500	5.9 ± 1.1 a	20.6 ± 7.6 a	56.8 ± 4.1 bc
	1,000	10.1 ± 7.1 a	25.2 ± 7.2 a	52.3 ± 2.8 c
	2,000	7.9 ± 3.2 a	20.6 ± 7.2 a	42.2 ± 6.2 d
CK		4.9 ± 1.6 a	5.5 ± 0.8 b	7.1 ± 2.4 e
Thiamethoxam 25% SG	100	59.1 ± 5.3 a	78.3 ± 2.2 a	96.9 ± 2.7 a
	200	49.8 ± 5.3 b	81.4 ± 7.5 a	96.8 ± 4.2 a
	500	49.5 ± 3.5 b	78.9 ± 4.6 a	97.4 ± 2.4 a
	1,000	41.6 ± 3.1 c	69.0 ± 5.4 b	97.8 ± 3.7 a
	2,000	39.5 ± 4.0 c	63.8 ± 2.6 b	95.1 ± 5.0 a
CK		4.1 ± 0.8 d	7.8 ± 1.5 c	8.8 ± 1.0 b

[z] Means among different rates of an insecticide followed by the same letter(s) are not significantly different at 5% level by Fisher's protected LSD test. Percentage data were subjected to arcsine-square-root transformation prior to analysis.

[y] Time after experiments initiated.

表 2. 抗生素類殺蟲劑與食餌以不同比例混合於非選擇性試驗中對瓜實蠅之平均死亡率。

Table 2. Mean mortalities of *Bactrocera cucurbitae* exposed to food baits incorporated with different dilution rates of antibiotic insecticides in no-choice tests.

Insecticide	Dilution rate (×)	% Cumulative mortality (Mean ± SD)[z] 24 h[y]	48 h	72 h
Abamectin 2% EC	100	33.3 ± 4.7 a	68.2 ± 3.6 a	88.3 ± 3.6 a
	200	27.2 ± 7.8 a	61.6 ± 14.0 a	84.9 ± 3.0 a
	500	31.7 ± 6.3 a	60.4 ± 10.8 a	85.9 ± 1.9 a
	1,000	30.4 ± 3.3 a	65.1 ± 7.1 a	86.1 ± 2.1 a
	2,000	28.7 ± 2.0 a	61.4 ± 5.5 a	72.9 ± 4.1 b
CK		1.5 ± 1.5 b	1.5 ± 1.5 b	1.5 ± 1.5 c
Emamectin benzoate 2.15% EC	100	69.2 ± 4.7 a	88.0 ± 3.7 a	94.6 ± 2.1 a
	200	61.4 ± 5.3 a	85.8 ± 6.7 a	93.4 ± 1.6 a
	500	60.8 ± 12.4 a	87.5 ± 3.0 a	95.2 ± 1.4 a
	1,000	62.6 ± 5.3 a	83.8 ± 3.3 a	92.4 ± 2.6 a
	2,000	59.9 ± 6.7 a	88.7 ± 2.3 a	94.9 ± 1.6 a
CK		0.0 ± 0.0 b	1.6 ± 1.6 b	4.8 ± 1.6 b
Milbemectin 1% EC	100	13.1 ± 2.3 a	29.8 ± 2.1 a	36.6 ± 4.0 a
	200	13.8 ± 1.6 a	29.8 ± 3.6 a	34.0 ± 4.4 a
	500	9.0 ± 1.7 ab	15.9 ± 5.4 b	18.0 ± 6.3 b
	1,000	4.3 ± 6.1 bc	8.5 ± 9.5 bc	9.0 ± 9.2 c
	2,000	3.2 ± 2.7 bc	3.7 ± 2.4 c	4.8 ± 1.6 c
CK		1.6 ± 1.6 c	2.6 ± 1.8 c	2.6 ± 1.8 c
Spinetoram 5.87% SC	100	78.6 ± 3.8 a	95.8 ± 4.8 a	99.5 ± 0.9 a
	200	71.1 ± 5.8 ab	93.9 ± 5.5 ab	99.5 ± 0.9 a
	500	71.3 ± 5.0 ab	87.5 ± 9.5 abc	98.4 ± 2.7 ab
	1,000	67.2 ± 4.2 bc	82.9 ± 5.2 bc	96.4 ± 1.8 b
	2,000	60.0 ± 1.4 c	84.8 ± 3.0 c	97.4 ± 1.7 ab
CK		1.0 ± 0.9 d	4.7 ± 1.6 d	7.9 ± 1.7 c
Spinosad 2.5% SC	100	65.5 ± 4.2 a	86.5 ± 8.7 a	95.3 ± 4.2 a
	200	55.7 ± 6.4 a	83.9 ± 14.6 a	94.6 ± 5.7 a
	500	42.5 ± 7.5 b	73.4 ± 8.9 a	90.9 ± 7.9 a
	1,000	40.3 ± 9.4 b	80.4 ± 15.4 a	92.8 ± 6.9 a
	2,000	32.9 ± 7.1 b	71.8 ± 5.4 a	88.6 ± 8.9 a
CK		1.0 ± 0.9 c	3.2 ± 1.6 b	3.2 ± 1.6 b
Spinosad 0.02% CB	0	7.6 ± 3.1 d	45.6 ± 14.2 b	68.2 ± 6.8 c
	1	43.2 ± 3.5 c	78.9 ± 5.9 a	91.4 ± 3.9 a
	2	51.1 ± 8.8 bc	78.7 ± 9.7 a	90.9 ± 4.1 a
	4	73.3 ± 5.5 a	86.5 ± 4.8 a	91.9 ± 1.1 a
	8	61.0 ± 9.0 b	78.5 ± 6.6 a	83.0 ± 5.9 b
CK		1.1 ± 1.0 e	2.7 ± 1.9 c	4.9 ± 1.6 d

[z] Means among different rates of an insecticide followed by the same letter(s) are not significantly different at 5% level by Fisher's protected LSD test. Percentage data were subjected to arcsine-square-root transformation prior to analysis.

[y] Time after experiments initiated.

表 3. 新尼古丁殺蟲劑與食餌以不同比例混合於選擇性試驗中對瓜實蠅之平均死亡率。

Table 3. Mean mortalities of *Bactrocera cucurbitae* exposed to food baits incorporated with different dilution rates of neonicotinoids in choice tests.

Insecticide	Dilution rate (×)	% Cumulative mortality (Mean ± SD)[z]		
		24 h[y]	48 h	72 h
Acetamiprid 20% SP	100	21.9 ± 3.9 a	43.1 ± 4.2 a	64.8 ± 5.7 a
	200	16.6 ± 8.3 ab	40.0 ± 9.4 ab	70.4 ± 2.4 a
	500	11.0 ± 4.2 b	27.2 ± 11.0 ab	64.4 ± 7.8 a
	1,000	11.0 ± 4.2 b	23.2 ± 14.2 b	63.1 ± 0.4 a
	2,000	9.0 ± 1.2 b	32.8 ± 1.9 ab	63.5 ± 4.2 a
CK		2.7 ± 2.5 c	4.3 ± 3.7 c	7.0 ± 5.0 b
Clothianidin 16% SG	100	43.4 ± 3.8 ab	69.9 ± 3.1 a	82.1 ± 2.4 a
	200	42.8 ± 3.2 ab	68.8 ± 0.5 a	82.0 ± 2.9 a
	500	50.8 ± 5.9 a	70.7 ± 2.6 a	84.3 ± 1.7 a
	1,000	39.1 ± 2.5 b	63.3 ± 4.0 a	81.7 ± 3.2 a
	2,000	25.8 ± 8.1 c	52.2 ± 6.6 b	66.9 ± 5.8 b
CK		0.0 ± 0.0 d	1.5 ± 1.5 c	1.5 ± 1.5 c
Dinotefuran 20% SG	100	40.3 ± 3.1 a	70.2 ± 0.8 a	83.7 ± 3.4 a
	200	39.9 ± 4.8 a	67.8 ± 3.3 a	83.7 ± 3.8 a
	500	33.1 ± 3.8 b	55.1 ± 5.9 b	73.0 ± 2.7 b
	1,000	24.5 ± 1.7 c	49.1 ± 4.3 b	62.4 ± 5.7 c
	2,000	26.0 ± 3.0 c	47.1 ± 6.2 b	61.5 ± 2.9 c
CK		0.0 ± 0.0 d	1.6 ± 1.5 c	1.6 ± 1.5 d
Thiamethoxam 25% SG	100	46.6 ± 2.6 a	68.5 ± 4.7 a	84.2 ± 2.1 a
	200	40.1 ± 6.1 ab	67.1 ± 3.5 a	84.0 ± 2.5 a
	500	38.8 ± 6.6 ab	63.3 ± 4.9 a	84.0 ± 4.7 a
	1,000	37.7 ± 4.3 b	67.0 ± 0.3 a	83.0 ± 3.3 a
	2,000	29.6 ± 1.3 c	51.5 ± 4.6 b	75.5 ± 1.9 b
CK		4.5 ± 0.9 d	5.7 ± 1.2 c	8.4 ± 1.4 c

[z] Means among different rates of an insecticide followed by the same letter(s) are not significantly different at 5% level by Fisher's protected LSD test. Percentage data were subjected to arcsine-square-root transformation prior to analysis.
[y] Time after experiments initiated.

率選擇性較非選擇性稍高，但所有含抗生素殺蟲劑的食餌在選擇性與非選擇性試驗間的死亡率相差皆不多，且其中可能含有不同試驗時間所造成的誤差 (表 4)。

討論

當食品安全與環境保護成為受到關注的重點時，農藥常常成為被詬病的主要對象。選擇低毒性的農藥並降低農藥直接噴施於作物上，同時讓農作物保有穩定的產量與品質，成為作物蟲害防治研究上的重要課題。利用餌劑進行果實蠅類害蟲的防治，可以降低農藥的使用量，進一步以高效低毒的殺蟲劑取代有機磷或胺基甲酸鹽類殺蟲劑作為誘餌添加藥劑，除了可降低農藥對環境的污染外，也可同時保障農民的健康 (Wang *et al*. 2013)。

本研究篩選出新尼古丁類殺蟲劑可尼丁、達特南和賽速安，以及抗生素殺蟲劑因滅汀、賜諾特和賜諾殺，添加於成蟲食餌中對瓜實蠅具有良好的毒殺效果。非選擇性試驗結果，雖然顯示上列殺蟲劑對瓜實蠅的毒殺速率較為緩

表 4. 抗生素類殺蟲劑與食餌以不同比例混合於選擇性試驗中對瓜實蠅之平均死亡率。

Table 4. Mean mortalities of *Bactrocera cucurbitae* exposed to food baits incorporated with different dilution rates of antibiotic insecticides in choice tests.

Insecticide	Dilution rate (×)	% Cumulative mortality (Mean ± SD)[z] 24 h[y]	48 h	72 h
Abamectin 2% EC	100	22.9 ± 1.0 b	53.0 ± 2.2 a	82.1 ± 5.6 a
	200	24.4 ± 11.6 ab	50.4 ± 12.7 a	80.6 ± 3.6 ab
	500	37.7 ± 1.7 a	63.9 ± 6.8 a	81.5 ± 4.4 a
	1,000	28.2 ± 9.2 ab	51.1 ± 11.7 a	70.5 ± 8.9 b
CK		1.0 ± 0.9 c	2.0 ± 0.9 b	2.0 ± 0.9 c
Emamectin benzoate 2.15% EC	100	58.3 ± 8.7 a	86.3 ± 4.9 a	92.1 ± 1.4 a
	200	62.0 ± 3.4 a	80.7 ± 7.5 a	89.8 ± 3.8 a
	500	60.6 ± 11.0 a	82.4 ± 4.4 a	90.5 ± 6.7 a
	1,000	54.6 ± 8.6 a	82.9 ± 8.8 a	88.1 ± 3.5 a
	2,000	54.9 ± 6.7 a	82.1 ± 3.8 a	88.6 ± 4.0 a
CK		0.0 ± 0.0 b	1.6 ± 1.6 b	3.8 ± 1.0 b
Spinetoram 5.87% SC	100	72.1 ± 5.6 a	94.2 ± 7.4 a	98.4 ± 2.7 a
	200	63.9 ± 2.0 ab	94.8 ± 5.4 a	97.4 ± 2.4 ab
	500	59.0 ± 6.0 b	84.9 ± 8.8 ab	90.6 ± 5.8 bc
	1,000	56.6 ± 11.4 b	74.9 ± 6.9 bc	92.2 ± 4.6 bc
	2,000	38.2 ± 5.5 c	64.0 ± 3.0 c	84.6 ± 7.3 c
CK		1.0 ± 0.9 d	4.2 ± 0.9 d	6.9 ± 0.9 d
Spinosad 2.5% SC	100	55.2 ± 7.3 a	77.7 ± 9.8 a	89.8 ± 8.5 a
	200	55.0 ± 4.0 a	77.4 ± 13.2 a	88.7 ± 8.9 a
	500	41.1 ± 4.9 b	66.4 ± 7.5 ab	84.0 ± 9.4 ab
	1,000	32.6 ± 3.9 bc	64.6 ± 10.2 ab	83.4 ± 6.6 ab
	2,000	24.9 ± 6.1 c	55.6 ± 6.4 b	71.1 ± 6.7 b
CK		1.0 ± 0.9 d	2.1 ± 0.9 c	3.7 ± 1.8 c
Spinosad 0.02% CB	0	10.2 ± 6.2 b	31.3 ± 5.0 b	39.1 ± 11.2 b
	1	67.0 ± 7.3 a	81.6 ± 6.7 a	85.8 ± 6.0 a
	2	75.1 ± 5.2 a	83.5 ± 1.6 a	88.7 ± 5.3 a
	4	74.4 ± 1.4 a	86.3 ± 2.7 a	90.0 ± 1.0 a
	8	73.0 ± 2.4 a	86.8 ± 5.1 a	89.9 ± 5.1 a
CK		1.1 ± 1.0 c	3.3 ± 1.6 c	4.4 ± 1.9 d

[z] Means among different rates of an insecticide followed by the same letter(s) are not significantly different at 5% level by Fisher's protected LSD test. Percentage data were subjected to arcsine-square-root transformation prior to analysis.

[y] Time after experiments initiated.

慢，新尼古丁類殺蟲劑於 24 h 的瓜實蠅死亡率為 50%，抗生素類殺蟲劑則為 60%，然而兩者於 72 h 後瓜實蠅的累積死亡率皆 95% 以上。抗生素類殺蟲劑對瓜實蠅的毒殺速率相較於新尼古丁類殺蟲劑為快，可能是因為新尼古丁類殺蟲劑對害蟲的主要殺蟲機制為胃毒性 (Tomizawa & Casida 2005)，而抗生素類殺蟲劑除了胃毒性外亦具接觸毒性 (Lasota & Dybas 1991; Chio 2011)，因此對瓜實蠅有較快的毒殺效率。此外，稀釋 8× 的 0.02% 賜諾殺濃餌劑為目前政府所推薦的東方果實蠅防治用誘殺餌劑，綜合選擇性與非選擇性試驗結果

顯示，降低其稀釋倍數不會進一步提升誘殺效率，而未經稀釋的賜諾殺濃餌劑 24 h 後對瓜實蠅的死亡率低於 10%，即使經過 72 h，累積死亡率也比任何倍數稀釋的餌劑低 15% 以上，這可能與餌劑成分在無水狀態下瓜實蠅不易取食有關 (Mangan *et al*. 2006)。

試驗期間瓜實蠅的死亡率，可由毒餌對蟲體產生毒性或蟲體對毒餌拒食產生飢餓所引起，同時提供瓜實蠅含毒與無毒食餌進行選擇性試驗，瓜實蠅可能會偏好選擇無毒食餌，導致選擇性試驗之死亡率比非選擇性試驗為低。然而，本研究中抗生素類殺蟲劑在此二種情況下對瓜實蠅的致死率，選擇性低於非選擇性試驗的差異多在 5% 以內，新尼古丁類殺蟲劑差異多在 15% 以內；加上無論是否有無毒食餌可供選擇，瓜實蠅的死亡率皆依時間增長而增加，顯示上述抗生素類殺蟲劑對瓜實蠅的忌避作用很有限，而新尼古丁類殺蟲劑忌避作用也不強烈，瓜實蠅仍然會取食毒餌。由此可證實，本研究中的瓜實蠅死亡率主要是由殺蟲劑的毒性所致，而非飢餓所引起。

餌劑對瓜實蠅的毒殺效果，除了取決於殺蟲劑的毒性外，還有賴餌劑的誘引力吸引瓜實蠅前來取食致死 (Roessler 1989)。本研究結果顯示，非選擇性試驗中對瓜實蠅 24 h 的毒殺效果，賜諾殺濃餌劑較自行調製的含賜諾殺食餌，在二者有效成分含量相似時高出約 20%，48 h 和 72 h 二者累積的瓜實蠅死亡率則相當。然而在選擇性試驗中賜諾殺濃餌劑對瓜實蠅的誘殺效果相較於賜諾殺食餌，在 24 h 時的死亡率高出 40%，至 48 h 時的死亡率仍高出 20%，要到 72 h 二者累積死亡率才相當。本研究用來混合殺蟲劑的食餌，為一般試驗用的瓜實蠅成蟲飼料，而賜諾殺濃餌劑為研發出的優良果實蠅類害蟲專用餌劑 (Mangan *et al*. 2006)，對瓜實蠅的誘引效果應遠優於一般飼料用食餌，因此造成瓜實蠅在有無毒食餌可供選擇的情況下，2 種餌劑誘殺效果間的差異性更顯懸殊。此外，本研究中所有測試的殺蟲劑，選擇性試驗的死亡率皆稍低於非選擇性試驗，但賜諾殺濃餌劑選擇性試驗的死亡率卻不比非選擇性試驗為低。由此亦顯示，良好的誘引劑配方可有效提升餌劑與相關食源的競爭力，在田間亦可發揮與天然寄主間的競爭潛力，對餌劑的誘殺效果具關鍵決定性。本研究中所測試的抗生素類殺蟲劑阿巴汀，試驗結果雖顯示對瓜實蠅缺乏快速的毒殺效果，要累積到 72 h 才可獲得較滿意的死亡率，然而未來若混合在合適的餌劑中，誘殺效果可能會有所提升，因此仍可列入瓜實蠅餌劑添加藥劑的考量之一。

防治技術的多樣化是害蟲綜合管理的重點之一，除了可提升防治效果外，亦可避免害蟲對藥劑產生抗藥性，增加藥劑的商品壽命。目前有研究顯示，果實蠅類害蟲對胺基甲酸鹽、有機磷、合成除蟲菊與賜諾特等類殺蟲劑可能都已經產生抗藥性 (Vontas *et al*. 2011)，因此尋找可供輪用的防治用藥劑也成為研究的重點。新尼古丁和抗生素類殺蟲劑相較於傳統化學性殺蟲劑，對瓜實蠅的毒效可能較為緩慢，但最終對瓜實蠅的死亡率仍與一些常用的化學性殺蟲劑相當 (Lai & Yang 2012; Lai 2013)，且具有低毒及對環境友善的優點。本研究結果亦顯示，測試的因滅汀和賜諾特對瓜實蠅的毒殺效率較目前推薦的果實蠅防治用餌劑之添加藥劑賜諾殺為快，而新尼古丁類殺蟲劑可尼丁、達特南和賽速安毒殺瓜實蠅的效率也與賜諾殺相當。因此，上述篩選出的藥劑非常值得進一步深入研究，探討其作為瓜實蠅防治用餌劑之添加藥劑的潛力。

引用文獻

Ansari, M. S., F. Hasan, and N. Ahmad. 2012. Threats to fruit and vegetable crop: Fruit flies (Tephritidae)-ecology, behaviour, and management. J. Crop Sci. Biotechnol. 15:169–188.

Chio, E. H. 2011. Spinosyn insecticides: Part I. Blockbuster products from a remarkable discovery. Formosan Entomol. 31:1–13. (in Chinese with English abstract)

Dhillon, M. K., R. Singh, J. S. Naresh, and H. C. Sharma. 2005. The melon fruit fly, *Bactrocera cucurbitae*: A review of its biology and management. J. Insect Sci. 5:40.

Fei, W. C., Y. C. Wang, F. S. Chen, S. M. Lin, and Y. H. Lee. 2010. Plant Protection Manual. Agricultural Chemicals and Toxic Substances Research Institute Press. Taichung, Taiwan. 963 pp. (in Chinese)

Ghanim, M. and I. Ishaaya. 2011. Insecticides with novel modes of action: Mechanism and resistance management. p.385–407. *in*: Tolerance to Environmental Contaminants. (Amiard-Triquet, C., P. S. Rainbow, and M. Roméo, eds.) CRC Press. Boca Raton, Florida. 446 pp.

Hsu, J. C. and H. T. Feng. 2002. Susceptibility of melon fly (*Bactrocera cucurbitae*) and oriental fruit fly (*B. dorsalis*) to insecticides in Taiwan. Plant Prot. Bull. 44:303–315.

Hsu, J. C., H. T. Feng, and W. J. Wu. 2004. Resistance and synergistic effects of insecticides in *Bactocera dorsalis* (Diptera: Tephritidae) in Taiwan. J. Econ. Entomol. 97:1682–1688.

Hsu, J. C., L. H. Huang, H. T. Feng, and W. Y. Su. 2015. Do organophosphate-based traps reduce control efficiency of resistant tephritid flies? J. Pest Sci. 88:181–190.

Lai, H. S. 2013. Lethal effects of organophosphates mixed with adult food against the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). J. Taiwan Agric. Res. 62:372–381. (in Chinese with English abstract)

Lai, H. S. and T. C. Yang. 2012. Effectiveness of bait incorporated with carbamates or pyrethroids against the melon fly. Bull. Hualien DARES 30:43–51. (in Chinese with English abstract)

Lasota, J. A. and R. A. Dybas. 1991. Avermectins, a novel class of compounds: Implications for use in arthropod pest control. Annu. Rev. Entomol. 36:91–117.

Mangan, R. L., D. S. Moreno, and G. D. Thompson. 2006. Bait dilution, spinosad comcentration, and efficacy of GF-120 based fruit fly sprays. Crop Prot. 25:125–133.

Roessler, Y. 1989. Control; insecticides; insecticidal bait and cover sprays. p.329–336. *in*: World Crop Pests 3(B). Fruit Flies; Their Biology, Natural Enemies and Control (Robinson, A. S. and G. Hooper, eds.) Elsevier. Amsterdam, The Netherlands. 372 pp.

Tomizawa, M. and J. E. Casida. 2005. Neonicotinoid insecticide toxicology: Mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45:247–268.

Vontas, J., P. Hernandez-Crespo, J. T. Margaritopoulos, F. Ortego, H. T. Feng, K. D. Mathiopoulos, and J. C. Hsu. 2011. Insecticide resistance in Tephritid flies. Pestic. Biochem. Physiol. 100:199–205.

Wang, B., Y. Yang, J. Huang, and J. Chen. 2013. Toxicity of pesticides in protein bait to *Bactrocera dorsalis*. J. South China Agric. Univ. 34:37–40. (in Chinese with English abstract)

Lethal Effects of Adult Food Incorporated with Neonicotinoids and Antibiotic Insecticides against the Melon Fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

Hsin-Shun Lai[1,*]

Abstract

Lai, H. S. 2016. Lethal effects of adult food incorporated with neonicotinoids and antibiotic insecticides against the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). J. Taiwan Agric. Res. 65(3):296–305.

The melon fly, *Bactrocera cucurbitae* (Coquillett), is an important pest of cucurbit crops. Application of poison baits is the major strategy recommended by the government for the melon fly control. This study was conducted to screen out effective neonicotinoids and antibiotic insecticides with no-choice (only a poisoned bait being provided) and choice (a non-poison bait being provided together with a poisoned bait) tests in the laboratory. It helps in providing important information for the selection of low-toxic insecticide additives for melon fly baits that reduces the harmful effects imposed on the environments and the farmers, as well as increasing the insecticide options to reduce the development of insecticide resistance. Results of no-choice tests showed that the neonicotinoids-thiamethoxam, clothianidin, and dinotefuran, and the antibiotic insecticides-emamectin benzoate, spinetoram, and spinosad added to the adult foods elicited high lethal effects to the melon flies. These selected neonicotinoids resulted in approximately 50% mortalities which was a little lower than 60% mortalities caused by the selected antibiotic insecticides. However, both insecticide groups all made the melon fly mortalities reached 95% at the end of 72-h experimental periods. Results also showed that the rates of melon fly mortalities induced by emamectin benzoate and spinetoram were faster than that induced by spinosad, a registered insecticide additive for fruit fly food baits, and the mortality rates by the thiamethoxam, clothianidin, and dinotefuran were similar to the spinosad. Choice tests indicated that both the selected neonicotinoids and antibiotic insecticides caused limited deterrent effects, but substantial insecticidal toxicities to the melon flies. Collectively, this study suggests that the above selected insecticides and abamectin, the one with a little less lethality, deserve further investigations for their potential as effective insecticide additive in the melon fly baits.

Key words: *Bactrocera cucurbitae*, Neonicotinoid, Aantibiotic insecticide, Bait.

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台灣農業研究 (J. Taiwan Agric. Res.) 65(3):306–312 (2016)
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研究報告

開頂溫室自動控制系統研發

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摘要

姚銘輝、洪福良、趙宗明。2016。開頂溫室自動控制系統之研發。台灣農業研究 65(3):306–312。

夏季高溫問題一直是溫室環境控制難以解決的問題，尤其是一般建置成本較低之蔬果溫室。本研究以文心蘭生長需求為依據，將溫室各項捲簾開閉之控制參數化，結合自動控制技術，以研發開頂溫室之自動控制系統。本研究之作物對象為文心蘭，文心蘭和其他作物相較，其生長環境於低光照狀態且可接受較高的環境溫度，所以在降溫策略的擬定上，可於較低光及較高溫的環境生長。研究結果顯示，開頂對於溫室內微氣象影響，可因垂直風速增加而將熱氣及水汽排於溫室外。另依據作物生理需求所規劃溫室控制流程，由實測結果，自動開頂控制系統有助於降低夏季溫室內積熱，未來可依據不同作物生理需求分別建立控制系統，以提升簡易溫室對於環境控制的能力。

關鍵詞：開頂溫室、自然對流、自動控制、微氣象。

前言

政府近年來積極推廣設施栽培，以調節產期、提升品質、促使產業由傳統產業轉型為精緻農業。10 年來設施栽培農業發揮較優勢之生產方式，創造較高經濟價值，但生產環境卻隨著氣候變遷使得作物生長面臨更多的逆境。雖然投入降溫設備後可有效營造適合作物生產的環境，但生產成本卻非蔬果類作物所能承擔，如何以低成本及運用氣象資源改善溫室環境是本研究之目的。擴大開頂面積以降溫，並經由垂直風速增加將溫室內高溫及高濕排出室外，皆是有效做法。另因設施栽培土壤缺少長期及大量的雨水淋洗，農民又慣於施用過量的肥料，在連續高密度複種情況下，經常會出現土壤因鹽類累積而劣化。改善溫室氣象環境，增加葉片蒸發散量以帶動蒸散流增加根部對水分及營養鹽的吸收，除增加肥料使用效率，也可減少土壤鹽分過度累積風險。

農業設施乃藉由人為方式來控制栽培環境，藉以達到提高產量、改善品質及穩定生產等目的。目前農業設施泛指以溫室 (greenhouses) 栽培之耕作制度，但就溫室的定義相當稀少，大部分以環境控制農業 (controlled environment agriculture; CEA) 的名詞出現的文獻較多 (Scott *et al*. 2005; Alec 2013)。環境控制農業，是指任何使農業生產者操控作物的環境，以達到作物生長需求條件之農業技術與設施。台灣地區溫室型式可概分為簡易型及精密型 2 種，精密型如蘭花溫室，是高投入及高成本之溫室栽培模式，可透過各項環境控境設備以生產高產值之農作物。另 1 種為種植蔬果所用之簡易型溫室，採用鍍鋅鋞管或輕型鋼材為材料，頂部以透光之塑膠布覆蓋，四周為塑膠布捲簾，捲簾內裝設防蟲網，鮮少需能源投入之設備，建置及營運成本相對低廉，是目前台灣溫室栽培之主流型式。然而，簡易型溫室在夏季面臨溫室內高溫及高濕之逆境，夏季溫度常超過 40℃，不僅作物無法生長，農民也

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難以在溫室內工作。由於夏季溫室高溫之熱源來自於太陽輻射穿透頂部塑膠布及部分輻射對塑膠布加熱之作用，使其熱源往溫室內傳送而造成高溫出現。在熱帶及亞熱帶國家之溫室栽培，夏季皆會面臨溫室內高溫問題。Kumar *et al*. (2009) 認為解決方法包括遮蔭降低太陽光輻射量，改變溫室結構以增加自然通風，研發塑膠布材質以改善光學特性，利用水汽 / 霧蒸發以吸收溫室內熱氣等。其中溫室開頂增加通氣面積以增加自然對流，是一種低成本之溫室降溫策略 (Baptista *et al*. 1999; Atarassi *et al*. 2006)。

開頂溫室對於作物生長的益處，在夏天將因開頂而減少溫室效應積熱問題，冬天則覆蓋增溫減少寒害發生，以維持溫室溫度的平穩。另可因垂直對流增加，水汽氣壓差增加，溫室作物葉片氣孔開啟，蒸散流加速，有助於根部營養吸收，也減少溫室土壤鹽化問題。開頂雖具有降低溫室內溫度及濕度作用，但因現有大部分開頂結構為固定型，為防止雨水濺入溫室內，一般開口小且開口方向與溫室垂直，如此熱氣及水汽無法快速散出，對於降溫效果有限。本文主要是研發 1 套開頂自動控制系統，環境控制項目包括頂部塑膠布、四周捲簾及外遮蔭，所有控制元項皆可由馬達驅動，並連結於控制器，再依據作物生長需求規劃控制程序，以進行溫室內微氣象控制。

材料與方法

溫室規格及設備

兩棟開頂溫室之規格，其結構為圓屋頂鋁管塑膠布溫室，長 12 m、寬 5 m，屋頂高度 4.3 m，集水槽高度 3 m，覆蓋材料為 0.18 mm 厚之塑膠膜。屋頂上方建置外遮蔭，四面周壁裝有 32 網目之防蟲網及捲揚作業的塑膠布，溫室屋頂為開頂型，由馬達驅動使塑膠布捲收至屋頂頂部。開頂面積 31.2 m^2，佔圓頂總面積 52%。環境控制項目包括頂部塑膠布、兩側捲簾及外遮蔭，皆可由馬達驅動，並由控制系統依據作物生長需求調整頂部與兩側捲簾捲簾及遮蔭網，進行溫室內微氣象之控制。

氣象環境偵測

儀器包括全天輻射儀 (pyranometer) (Eppley precision spectral pyranometer; PSP)；光度計 (quantum sensors) (Li-190SA)，測定範圍 400–700 nm，每一感測器在使用前以光學輻射校正器 (optical radiation calibrator) (Licor 1800-02) 校正參數；戶外大氣所使用之溫濕度計 (hygro-thermometer) (Campbell CS 500)；溫室內感測器裝設位置示意圖，如圖 1

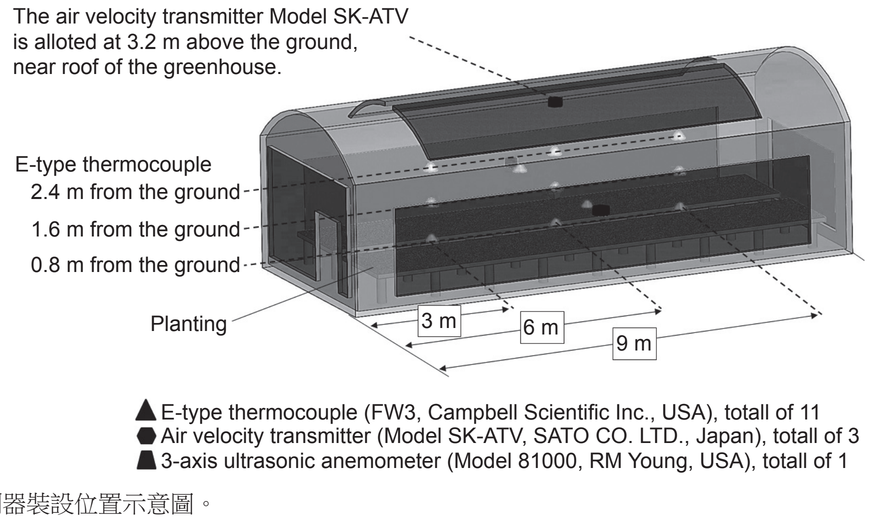

圖 1. 感測器裝設位置示意圖。

Fig. 1. Sensing elements for an automatic open-roof greenhouse.

所示。E-type 熱電偶溫度計 (FW3, Campbell Scientific Inc., USA)，共 11 支，分別在距地面 0.8、1.6 與 2.4 m 處的前、中、後各擺放 3 支，另外 2 支則在溫室內左右兩側，藉以監測整間溫室空間的溫度分布情形，輸出規格為 60 μV ℃$^{-1}$。熱線風速計 (Model SK-ATV, SATO Co. Ltd., Japan)，共 3 支，測定範圍為風速 0–30 m s^{-1} (溫度補償：0–60℃)，測定精度為風速 ± 3% FS，分別置於兩側牆面通風之防蟲網與上方接近開頂通風口處，主要為監測大氣入口風速與風向。三維音波風速計 (Model 81000, RM Young, USA)，共 1 支，風向分辨率 0.1 向，風速分辨率 0.01 m s^{-1}，置於溫室空間最中央的位置，藉以觀測整間溫室三維的風速與風向。我們將上述感測器搭配資料抓取裝置 (CR1000, Campbell Scientific, USA)，資料擷取間隔設定為每筆 1 min，擷取時間設單日為 00:00–23:59 之間共 14,400 個分析點，不同的實驗溫室測試條件則分不同天進行。

光合作用速率及蒸散速率量測

利用光合作用分析儀 (Model 6400 Licor, Inc., Lincoln, NE, USA) 量測作物葉片光合作用速率、蒸散速率、氣孔導度及氣孔內腔二氧化碳濃度 (Ci) 之數值。

開頂溫室自動控制策略

將外遮蔭、頂部塑膠布、前後側捲簾及左右側捲簾共 4 種降溫設備之馬達啟動控制，連結於以資料收集器為主體之自動控制系統，控制程序為控制 10 min 動作 1 次。第一優先：下雨時頂部關閉。溫度大於 26℃時，則頂部、左右側側、前後側捲簾開啟。溫度小於 24℃時，則頂部、左右側捲簾關閉，溫度小於 20℃，則前後、左右側捲簾再關閉。光度大於 500 μmol，則外遮蔭開啟，光度小於 100 μmol，則外遮蔭關閉。

結果與討論

溫室環境的設定來自於作物生理需求，每種作物有其最佳生育條件，包括對溫度、濕度、光照及水分的需求，溫室內能夠控制之氣象因子以溫度及光度為主。有關於蝴蝶蘭光合作用特性及與氣象因素 (尤其是溫度及光度) 的交感已有相當多研究 (Yao *et al*. 2011)，對於推估蝴蝶蘭園之最佳溫度及光照條件，已有較詳細的統計數據與實驗條件。我們參照文獻參數，並利用環控試驗室量測不同溫度及光度對於文心蘭葉片之光合作用影響程度。由圖 2 的結果，說明文心蘭對於光度需求及敏感度較低，當光量子密度 (photosynthetic photon flux

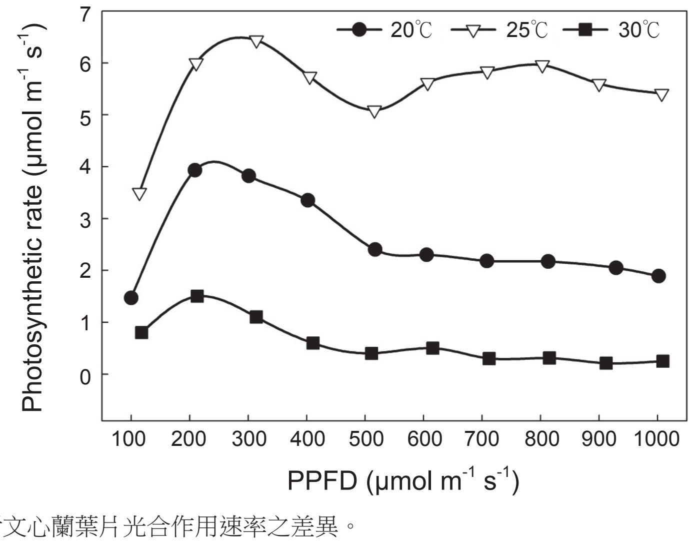

圖 2.　溫度及光度對文心蘭葉片光合作用速率之差異。

Fig. 2.　The changes of ptotosynthesis rates of Oncidium leaf under the different temperatures and PPFD combinations.

density; PPFD) 在 200–300 μmol m^{-2} s^{-1} 即達飽和點，但溫度效應則影響極大；25℃有最高光合作用速率，約可達 6 μmol m^{-2} s^{-1}，20℃為 4 μmol m^{-2} s^{-1}，溫度高於 30℃在適當光度下則只剩下 1.5 μmol m^{-2} s^{-1}。由此結果，說明文心蘭對溫度敏感遠高於光度，光度控制在 200–300 μmol m^{-2} s^{-1} 即可達最佳條件。另由實測溫室內文心蘭之生理參數，將光合作用儀設定為自動偵測模式，每 10 min 偵測 1 次，圖 3 為葉片生理參數及即時氣象在 1 日間之變化。當太陽光在 6:00 開始照射後，文心蘭葉片之光合作用及蒸散作用開始啟動，顯然此時氣孔開啟，葉片進行固碳作用。並隨光度逐漸增加，文心蘭葉片對二氧化碳羧化反應提高，但當葉片溫度到達 30℃，此時光度約為 150 μmol m^{-2} s^{-1}，光合作用及蒸散作用達到最高 (分別為 2.5 μmol CO_2 m^{-2} s^{-1} 及 0.4 μmol H_2O m^{-2} s^{-1})。之後葉溫皆高於 30℃，葉片生理反應停滯，一直到光度消失為止皆然。雖然這段時間有適宜之光度範圍 (200–300 μmol m^{-2} s^{-1})，然因溫度使氣孔關閉，而停止生理作用。這些基本作物葉片生理資料是開頂溫室自動控制決策的依據。

溫室增加通氣面積，可加大換氣率以減少因溫室效應之積熱問題 (Flores-Velazquez *et al*. 2014)，此種方式不需要動能也不需要後續溫室營運成本。當然，也因無降溫設備，所以溫室內溫度最佳狀態為與室外溫度相同。過去我國已有開頂類型之溫室，但因考量颱風問題，一般開口甚小或與溫室垂直，造成降溫效果不明顯。本研究所研發之自動開頂系統，主要是擴大開頂面積，讓熱氣及水氣直接向頂部傳送，較冷空氣由側邊進入，形成空氣循環。圖 4 為兩棟開頂溫室之外觀及控制設備，由馬達驅動外遮蔭，頂部捲簾及四周捲簾。

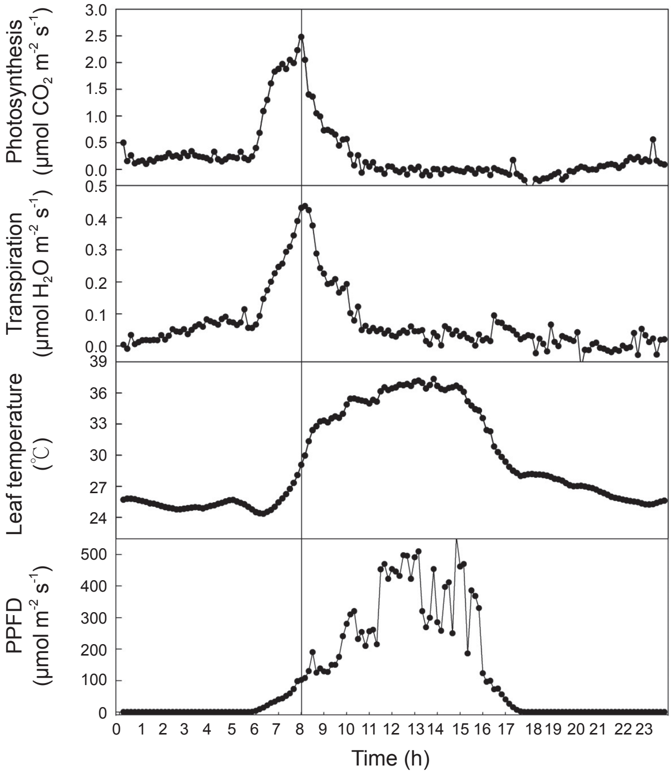

圖 3. 溫室內文心蘭葉片光合作用、蒸散作用、葉片溫度及光度之日變化。

Fig. 3. Diurnal changes of photosynthesis, transpiration, and temperature of an Oncidium leaf and fluctuation of PPFD in greenhouse.

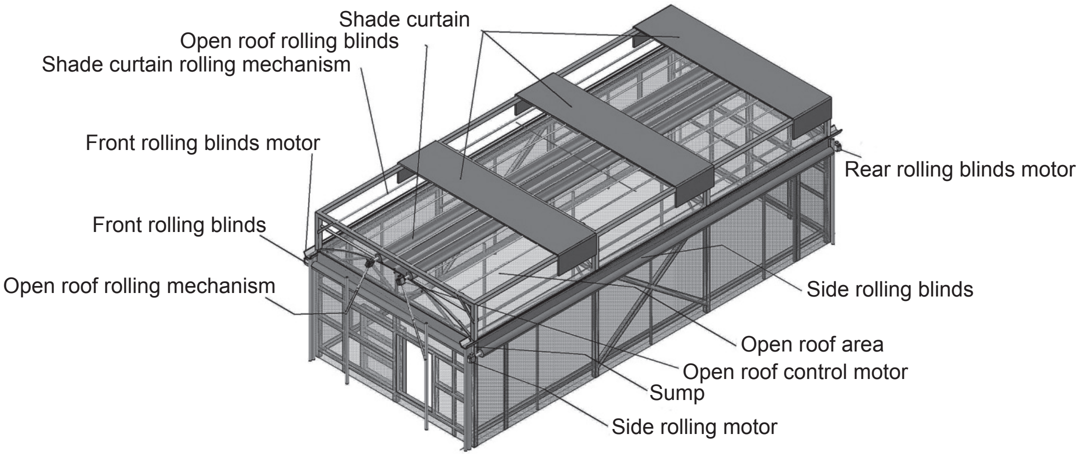

圖 4. 自動開頂溫室之控制設備配置情形。

Fig. 4. Control units for an automatic open-roof greenhouse.

為了解開頂結構對於溫室內微氣象的影響，在兩間溫室內各架設包括垂直及水平剖面之溫度計 11 支，熱線風速計 3 支，三維風速計 1 支及光度計 1 支，以提供溫室微氣象量測之用。其中，感測器裝設位置於圖 1 所示，同時以四周捲簾開啟為基本條件，一棟將開頂打開，另一則關閉，以觀測開頂對於溫室微氣象之影響。圖 5 為兩棟溫室之日溫度比較，開頂對於降溫效應，在正午 (11:00–13:00) 之間，每 1 min 擷取 1 筆資料，兩間溫室分別各有 120 個資料點，結果顯示約有 2–3℃的溫差，平均值 2.43℃，標準差為 1.45℃；濕度則差異相當明顯 (圖 6)，開頂在白天有助於水汽對外溢散，相對濕度較未開頂處理為低；開頂溫室之溫度及濕度較未開頂溫室為低，正午因煙囪效應 (stack effect) 是原因之一，以熱線風速計量測由地面向上之垂直風速變化 (圖 7)，垂直風速增加以帶動熱空氣及水汽的向上傳送，經頂部開啟而傳於室外。

由溫室實測結果說明，開頂對於降溫及除濕具有效果，但另一降溫方式為遮蔭。本研究在溫室頂端上約 50 cm 加裝自動外遮蔭系統，外遮蔭可降低太陽光對溫室塑膠布的加熱，減少溫室積熱產生，對於降溫具有更多效率。然而對於作物生長而言，遮蔭則減少葉片行光合作用，如何在遮蔭降溫及作物生長間取得平

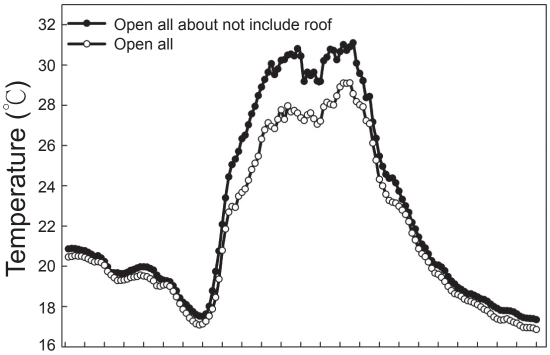

圖 5. 溫室內開頂及未開頂之日溫度變化。

Fig. 5. Diurnal variations of air temperature inside the open-roof and non open-roof greenhouses.

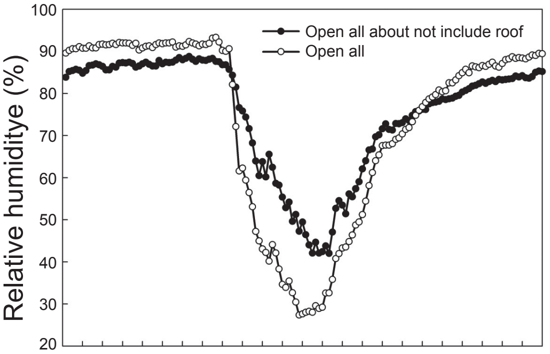

圖 6. 溫室內開頂及未開頂之日濕度變化。

Fig. 6. Diurnal variations of relative humidity inside the open-roof and non open-roof greenhouses.

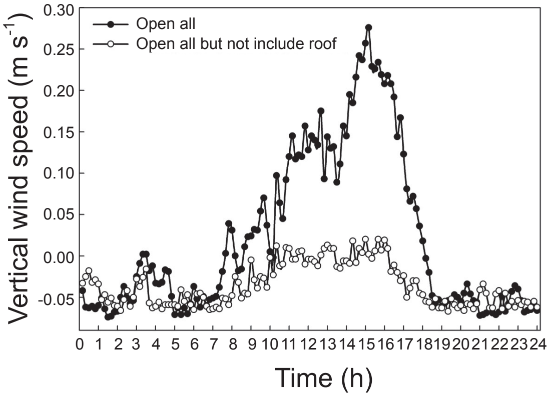

圖 7. 溫室內開頂及未開頂之垂直風速變化。

Fig. 7. Diurnal variations of vertical wind speed inside the open-roof and non open-roof greenhouses.

衡，需依作物生理特性作決定。由圖 2 及圖 3 可知，文心蘭和其他作物相較，可於較低光及較高溫的環境生長，所以在降溫策略的擬定上，採用本研究之自動化控制策略，依照作物生理訊號進而有效供應作物所需之環境狀態。圖 8 為由夏季開頂溫室自動控制系統實際運作下溫室內外之溫度差，由資料可知，在此控溫策略下，並且無循環風扇或使用其他耗能的降溫設備，開頂溫室可維持與戶外相近的溫度，皆在 ± 1.5℃以內。表示溫室內外空氣通風能充分交換，已有效避免溫室在夏季高溫下積熱之問題。

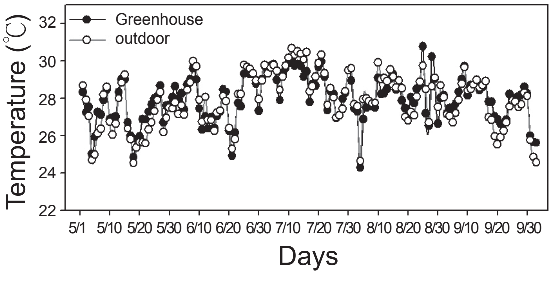

圖 8. 自動控制開頂溫室之夏季溫度變化。

Fig. 8. The daily average temperature of an auto-controlled greenhouse during the summer time (from May to September, 2012).

結論

本文除研究開頂對溫室微氣象改善之影響外，也同時研發 1 套自動控制系統，整合各項降溫及增加溫室通氣率之設備，如四周及頂部帷幕控制，外遮陰開閉等，以自然通氣方式降低溫室內積熱問題。而由實測結果，開頂對於溫室降溫效應，雖然由夏季日均溫可和室外相近，但在正午 (11:00–13:00) 時仍會高於室外溫度約會高 2℃；相對濕度的結果顯示，無開頂溫室平均相對濕度約有 43.1%，開頂溫室平均相對濕度約有 33.2%，兩者相差 9.9%。由此可知，開頂在白天是有助於水汽對外溢散，溫室內相對溼度低表示作物更容易進行蒸散作用，作物透過蒸散流以加速根部吸收水分 (及肥分)，有助於調整作物水分平衡及加速生長。本研究以文心蘭之生長需求為案例，導引出研發自動控制系統的動機，而由實測結果，自動開頂控制系統的利用是有助於降低夏季溫室因溫室效應所導致之積熱問題，未來可依據不同作物生理需求分別建立控制系統，以提升簡易溫室對於環境控制的能力。

引用文獻

Alec, M. 2013. The future of controlled environment agriculture. Appl. Eng. Agric. 20:13.

Atarassi, R. T., M. V. Folegatti, and R. P. C. do Brasil. 2006. Convection regime between canopy and air in a greenhouse. Sci. Agric. 63:77–81.

Baptista, F. J., B. J. Bailey, J .M. Randall, and J. F. Meneses. 1999. Greenhouse ventilation rate: Theory and measurement with tracer gas technique. J. Agric. Eng. Res. 72:363–374.

Flores-Velazquez, J., J. I. Montero, E. J. Baeza, and J. C. Lopez. 2014. Mechanical and natural ventilation systems in a greenhouse designed using computational fluid dynamics. Intl. J. Agric. Biol. Eng. 7:1–16.

Kumar, K. S., K. N. Tiwari, and M. K. Jha. 2009. Design and technology for greenhouse cooling in tropical and subtropical regions: A review. Energ. Buildings 41:1269–1275.

Scott, N. R., C. J. Rutzke, and L. D. Albright. 2005. Energy conversion options for energy-efficient controlled environment agriculture. HortScience 40:287–292.

Yao, M. H., C. J. Chen, and G. S. Huang. 2011. Estimating optimum temperatureand light intensity for orchid ofphalaenopsis from themeasurements of carbon dioxideflux and daily carbon balance. Crop Environ. Bioinform. 8:172–180. (in Chinese with English abstract)

Development of an Automatic Control System for Open-Roof Greenhouse

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Abstract

Yao, M. H., F. L. Hong, and C. M. Chao. 2016. Development of an automatic control system for open-roof greenhouse. J. Taiwan Agric. Res. 65(3):306–312.

Heat dissipation is a troublesome problem to solve for designing environmental control in a greenhouse, particularly for low-cost facilities in the summer. This study was conducted to develop an automatic controlling system for open-roof greenhouse in order to meet the demand for growing Oncidium. The on/off control of curtain will be parameterized and automatically controlled to provide the environment suitable for the growth of Oncidium, an orchid species that can tolerate more warm and humid conditions as well as lower illumination status than other crops. These characteristics are used in strategic planning of the cooling system. Results showed that the microclimate inside greenhouse can be manipulated in open-roof adjustment. The vertical wind speed was accelerated to drive out warming water vapor more effectively and efficiently so as to exhaust the hot air outside greenhouse. The temperature inside greenhouse may be lowered down by about 3℃ at noon in the summer. Results also showed that the controlling processes based on plant physiological mechanism can be reached with the designed system. With that, it further improves the applicability for controlling the greenhouse environment less costly.

Key words: Open-roof greenhouse, Natural ventilation, Automatic control, Micrometeorology.

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研究報告

經九年水稻玉米輪作下不同施肥管理對土壤氮和磷劃分之影響

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摘要

周恩存、王鐘和、鍾仁賜、陳琦玲。2016。經九年水稻玉米輪作下不同施肥管理對土壤氮和磷劃分之影響。台灣農業研究 65(3):313–327。

氮與磷是作物栽培上需要不斷補充之養分，然有許多因子會影響氮與磷在土壤中的轉變與有效性。本研究目的在探討施用不同種類之肥料在水 (水稻) 旱 (玉米) 田輪作下，經 9 年後對土壤中氮與磷劃分之影響。試驗共有 7 種施肥處理，分別為：(1) 未施肥之對照組 (CK)、(2) 化學氮肥區 (Chem-N)、(3) 堆肥區 (Comp)、(4) 堆肥與化學氮肥區之化學氮肥 1/3 用量共用區 (Comp + 1/3 N)、(5) 堆肥與化學氮肥區之化學氮肥 2/3 用量共用區 (Comp + 2/3 N)、(6) 綠肥與化學氮肥區之化學氮肥 1/3 用量共用區 (GM + 1/3 N)、(7) 泥炭與化學氮肥區之化學氮肥 1/3 用量共用區 (Peat + 1/3 N)。經栽培作物 9 年後，採取土壤以 Bremner (1965a, 1965b, 1965c) 的方法將氮劃分，以 Sui *et al*. (1999) 之序列萃取法將磷劃分。結果顯示，化學肥料施用對土壤，總氮與有機態氮無增加之效應，但卻使可水解氮濃度與占總氮的比例上升；堆肥與泥炭增加土壤總氮與有機態氮，且可水解氮與不可水解氮亦增加；與堆肥共用而多施之化學氮肥也無增加土壤總氮與有機態氮之作用；綠肥可緩慢提高土壤總氮與可水解氮。堆肥能增加可水解氮劃分之胺基酸態氮與可水解銨態氮濃度，其餘施肥處理對不可辨認的可水解氮及胺基醣態氮濃度之影響則較小。施磷肥使磷劃分分佈趨勢改變，留存之肥料磷主要轉變成碳酸氫鈉萃取無機磷與氫氧化鈉萃取無機磷。磷肥施用量多時，此兩劃分相對也增加較多，但有機質之性質會改變磷肥施用之影響，殘餘磷與鹽酸萃取磷受施肥管理之影響則較小。

關鍵詞：可水解氮、不可水解氮、碳酸氫鈉溶液可萃取磷、鹽酸溶液可萃取磷。

前言

土壤有機質的含量與特性影響土壤之物理、化學與生物性質，土壤有機質也是土壤品質之重要指標之一 (Doran *et al*. 1996; Reeves 1997)，而耕耘使土壤有機質含量下降 (Fließbach *et al*. 2007)。因此，適當的施用有機質肥料，乃今日農業上重要的施肥管理手段。不同有機質肥料組成各異 (Lee & Lian 2004)，在土壤中的降解、礦化速率亦不同 (Hadas & Pornoy 1994; Eghball *et al*. 2002; Lin *et al*. 2003)，大部分施入土壤中的有機質會在短時間被分解 (Rasmussen & Collins 1991; Darwish *et al*. 1995)，故土壤要累積有機質需要長期不斷補充。

土壤有機質是土壤中植物所需的許多元素的重要來源，尤其是氮與磷 (Bauer & Black 1994)，其化學型態影響其對植物的有效性。土壤有機質亦是土壤中微生物的能源，影響微生物活性，進而影響土壤中許多物理、化學

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與生物反應。因此，有機質肥料之施用對土壤各種性質之影響，與單施用化學肥料是不同的 (Kaur *et al*. 2005; Masto *et al*. 2006)，而不同的有機質種類影響亦不同。

土壤中的氮大部分為有機態 (Warren & Whitehead 1988; Kelley & Stevenson 1995)，施入土壤的有機質會影響土壤供氮能力，亦影響其在土壤中的累積量。施入土壤中的無機氮肥也會影響土壤氮的劃分 (fraction)，如利用 N-15 的研究顯示，第一生長季結束後約有 1/3 的化學肥料氮轉變為有機態，其中僅有少部分 (< 15%) 可在第二季被植物吸收 (Allen *et al*. 1973)。施肥量、作物移去量、土壤特性與氣候條件是影響有機與無機肥料磷在土壤中不同貯存庫分布之主要因子，McKenzie *et al*. (1992) 及 Schmidt *et al*. (1996) 之研究顯示，施用有機與無機肥料磷多年後，土壤中無機與有機磷均增加。

Bremner (1965a, 1965b, 1965c) 提出根據氮化合物的特性劃分 (fractionation) 土壤氮的方法，是廣被接受的土壤氮組成劃分法。Chang & Jackson (1957) 提出土壤磷的序列劃分，即用不同的萃取劑，將土壤中的磷按照化學特性劃分；Hedley *et al*. (1982) 則基於植物有效性的觀點發展序列劃分法。此 2 種磷劃分法經修改後 (Williams *et al*. 1967; Tiessin *et al*. 1984; Sui *et al*. 1999)，廣泛應用於土壤磷化學的研究。

國內對於農田土壤氮與磷的有效性研究甚多 (Tseng & Wang 1959, 1967; Chen 1971; Lee 1971; Lin *et al*. 1973a, 1973b; Lin *et al*. 1977; Lian *et al*. 1996)，然對土壤中氮與磷不同的化合物組成之研究卻相對較少。Lin *et al*. (1973a) 探討台灣主要水田土壤之氮劃分，顯示水田土壤氮中，不可水解氮 (nonhydrolyzable N) 占 24%，可水解全氮 (total hydrolyzable N) 占 76%，而可水解全氮中，胺基酸態氮 (amino acid-N)、可水解銨態氮 (hydrolyzable NH_4-N)、胺基醣態氮 (amino sugar N) 與不可辨認之可水解氮 (unidentified hydrolysable N) 分別占總氮之 36、25、5 及 10%。Lin *et al*. (1973b) 以 Bremner (1965a, 1965b, 1965c) 的氮劃分法與 Chang & Jackson (1957) 的磷劃分法，分別探討經 48 年連用相同肥料之水田土壤之氮與磷劃分，顯示不可水解氮平均占總氮的 36%，可水解氮占總氮 64%，而可水解氮中，胺基酸態氮、可水解銨態氮、胺基醣態氮及不可辨認之可水解氮分別占總氮之 31、16、6 及 17%；而各處理之各種可水解氮劃分占總氮的比例不同，顯示施肥管理會影響水田土壤氮劃分組成。Olk *et al*. (1996) 表示經 30 年集約之水稻栽培，使土壤中不穩定的腐質酸劃分 (labile humic acid fractions) 發生改變；而 Keeney & Bremner (1964) 之研究則顯示不單是總氮含量，不同劃分氮的分佈亦受耕作管理影響，如胺基酸態氮在草原土壤高於耕作土壤；Lin *et al*. (1973b) 磷之研究則顯示不同施肥管理之水田經 48 年後，其土壤平均鈣結合態磷、鋁結合態磷、鐵結合態磷分別占總磷之 15、31 及 23%，其研究亦顯示施肥管理會影響水田土壤磷劃分組成。

氣候條件 (Tiessen *et al*. 1984)、土壤性質 (Agebenin & Tiessen 1994) 及施肥種類與時間等耕作條件，均顯著影響土壤氮與磷型態 (Asami 1971; Allison 1973; Richards *et al*. 1995; Xu *et al*. 2003)。因此，由長期不同施肥管理後土壤氮與磷劃分的變化，可瞭解施肥管理和土壤氮與磷供應的關係。

長期試驗可以評估農業系統永續性與決定作物栽培對土壤之影響。台灣有關不同有機質肥料和化學肥料共用、單用化學肥料或單用有機質肥料，於長期輪作下對土壤氮與磷劃分的影響之研究仍鮮少。因此，本研究旨在探討水旱田輪作下，經過 9 年不同的肥培管理對土壤氮與磷劃分的影響，以作為土壤營養管理之參考。

材料與方法

地點與試驗設計

試驗土壤取行政院農業委員會農業試驗所 (Taiwan Agricultural Research Institute) 永續農法試驗田 (35 號田) (24°1.8' N, 120°41.4' E)，屬砂頁岩非石灰質新沖積土 (Fluvaquen-

tic Dystudept)，質地為壤土。試驗前土壤之 pH 值為 5.3 (1：1 土水比)、Bray-1 可萃取磷 14.8 mg kg^{-1}、有機碳 12.5 g kg^{-1}、總氮 1.1 g kg^{-1}；土壤之陽離子交換容量 (cation exchange capacity) 為 7.4 cmol k^{-1} (1.0 N neutral ammonium acetate)，交換性鉀、鈣與鎂分別為 74、625 與 149 mg kg^{-1}。

試驗自 1995 年 8 月開始進行，試驗田面積為 0.5 ha。本試驗樣品為經 9 年、水稻與玉米各栽培 9 期作之土壤，第 18 期作之作物為水稻。於 2004 年 6 月第 18 期作作物收穫後採集表層 (0–20 cm) 土壤，每小區採 4 個亞樣品組成一複合樣品。

試驗作物為春作水稻 (*Oryza sativa* L., cultivar 'TNG 67') 及秋作玉米 (*Zea mays* L., cultivar 'TNG 1')。使用之肥料為化學肥料與有機質肥料。化學肥料為硫酸銨 (種植水稻時施用)、尿素 (種植玉米時施用)、過磷酸鈣與氯化鉀；有機質肥料則為豬糞堆肥、綠肥 (埃及三葉草 *Trifolium alexandrinum* L. 或田菁 *Sesbania roxburghii* Merr.) 及泥炭。試驗共設定 7 種施肥處理如表 1：(1) 未施肥之對照組 (代號 CK)、(2) 化學氮肥區 (代號 Chem-N)、(3) 堆肥區 (代號 Comp)、(4) 堆肥與化學氮肥區 1/3 氮肥用量共用區 (代號 Comp + 1/3 N)、(5) 堆肥與化學氮肥區 2/3 氮肥用量共用區 (代號 Comp + 2/3 N)、(6) 綠肥與化學氮肥區 1/3 氮肥用量共用區 (代號 GM + 1/3 N)、(7) 泥炭與化學氮肥區 1/3 氮肥用量共用區 (代號 Peat + 1/3 N)。化學氮肥區肥料三要素 (N-P_2O_5-K_2O) 之施用量於種植玉米時為 140-80-80 kg ha^{-1}，種植水稻時為 120-60-60 kg ha^{-1}，其餘處理施氮量與 9 年之總施肥量如表 2。每處理 4 重複，逢機完全區集排列，小區面積為 20 × 6 m^2。各小區之位置及處理在試驗期間均維持不變。除未施肥之對照組外，其他處理均施用與化學

表 1. 各處理之氮肥施用量。
Table 1. Nitrogen application rate of different treatments.

	Application rate (kg ha^{-1})	
Treatment	Corn	Rice
CK	0 (without fertilization)	0 (without fertilization)
Chem-N	140 (urea N)	120 (ammonium sulfate N)
Comp	140 organic N (as compost)	120 organic N (as compost)
Comp + 1/3 N	140 (compost N) + 47 (urea N)	120 (compost N) + 40 (ammonium sulfate N)
Comp + 2/3 N	140 (compost N) + 93 (urea N)	120 (compost N) + 80 (ammonium sulfate N)
GM + 1/3 N	140 (green manure N) + 47 (urea N)	120 (green manure N) + 40 (ammonium sulfate N)
Peat + 1/3 N	140 (peat N) + 47 (urea N)	120 (peat N) + 40 (ammonium sulfate N)

表 2. 各處理 9 年之總施肥量。
Table 2. Total amounts of fertilization of different treatments during nine years.

Treatment	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
CK	0	0	0
Chem-N	2,340	550	1,050
Comp	2,340	2,254	1,590
Comp + 1/3 N	3,120	2,254	1,590
Comp + 2/3 N	3,900	2,254	1,590
GM + 1/3 N	3,120	892	3,950
Peat + 1/3 N	3,120	790	1,350

肥料區相同量的化學磷肥與鉀肥。玉米與水稻之磷肥均以基肥施入土壤中，玉米之鉀肥 50% 當基肥，50% 於播種後 30 d 追施；水稻之鉀肥 60% 當基肥，40% 於移植後 20 d 追施。

採樣與一般化學分析

採樣時，每小區採取 3 個亞樣品 (subsample)，組成一個複合樣品 (composite)。經風乾、磨碎並過 20 mesh 之篩網，置於夾鏈袋中儲存，並進行化學分析。分析酸鹼度 (土水比 1 : 1) (McLean 1982)、飽和水導電度 (Rhoades 1982)、有機碳以有機碳分析儀測定 (Model 1010 O.I. Analytical, Texas, USA)、總氮濃度 (Bremner 1965a, 1965b, 1965c)、Bray 1-P (Olsen & Sommers 1982)、Mehlich III 可萃取之鉀、鈣、鎂、銅與鋅 (Mehlich 1984)。

氮的分析

總氮 (Bremner 1965a, 1965b, 1965c)：秤取 1.0 g 土壤置於分解管，加入 2 mL 去離子水靜置 30 min，加入 1.1 g 分解促進劑 (硫酸鉀：硫酸銅：硒 = 100：10：1)、0.2 g 水楊酸及 8 mL 濃硫酸，靜置過夜。加 0.3 g 硫代硫酸鈉 ($Na_2S_2O_3$)，在 350℃下分解，分解後以蒸餾法定量。

無機態氮 (銨態氮 + 硝酸態氮) (Bremner 1965a, 1965b, 1965c)：5 g 土壤，以 50 mL 的 2.0 M 氯化鉀溶液萃取 2 h，過濾，以蒸餾法定量。

有機態氮劃分 (Bremner 1965a, 1965b, 1965c)：經無機態氮萃取後，土壤加 20 mL 6 N 鹽酸溶液，在 110–120℃下煮沸、迴流 (reflux) 12 h，得土壤水解液 (soil hydrolysate)，再測定各型態氮。

可水解全氮 (total hydrolyzable N)：適量中和過之水解液於分解管中，加入分解促進劑及濃硫酸分解，完成後，以蒸餾法定量。(1) 可水解銨態氮 (hydrolyzable ammonium-N)：適量水解液，加入過量氧化鎂 (MgO)，以蒸餾法定量。(2) 可水解銨及胺基醣態氮 (hydrolyzable ammonium-N + amino sugar-N)：適量水解液，加入磷酸鹽 - 硼酸鹽緩衝液 10 mL，以蒸餾法定量。(3) 胺基醣態氮 (amino sugar-N)：可水解銨及胺基醣態氮之值減去可水解銨態氮。(4) 胺基酸態氮 (amino acid-N) (Horwitz 1980)：適量水解液以茚滿三酮 (ninhydrin) 試劑呈色，比色法定量。(5) 不可辨認的可水解氮：可水解全氮減去可水解銨態氮、胺基醣態氮及胺基酸態氮。

不可水解氮：總氮減去可水解全氮與無機態氮。

磷的分析

以 Sui *et al.* (1999) 序列萃取劃分磷：(1) 0.5 g 風乾土置於 30 mL 的離心瓶中，加入 28 mL 的去離子水，振盪 16 h，2,862× *g* 離心 30 min，濾膜 (0.45 μm) 過濾，以鉬藍法測磷 (Murphy & Riley 1962)，為水萃取磷 (H_2O-P)。(2) 加入 25 mL 0.5 M pH 8.2 之碳酸氫鈉溶液於上述沉澱土壤之離心管中，振盪 16 h，2,862× *g* 離心 30 min，濾膜 (0.45 μm) 過濾，以鉬藍法測磷，為碳酸氫鈉萃取無機磷 ($NaHCO_3$-IP)。(3) 加入 25 mL 0.1 M 氫氧化鈉溶液於上述沉澱土壤之離心管中，振盪 16 h，2,862× *g* 離心 30 min，濾膜 (0.45 μm) 過濾，以鉬藍法測磷，為氫氧化鈉萃取無機磷 (NaOH-IP)。(4) 加入 25 mL 0.1 M 鹽酸溶液於上述沉澱土壤之離心管中，振盪 16 h，2,862× *g* 離心 30 min，濾膜 (0.45 μm) 過濾，以鉬藍法測磷，為鹽酸萃取磷 (HCl-P)。(5) 將剩餘土壤乾燥後取出移入分解管中，加入 1 mL 1 N 氯化鎂溶液、5 mL 濃硫酸，360℃下加熱分解 1 h，放置冷卻，加入 1 mL 混合酸 (濃硫酸：70–72% 過氯酸 = 92：2)，360℃加熱 5 min 取出定量至 50 mL，以鉬藍法測磷，為殘餘磷 (residual-P)。(6) 分別取碳酸氫鈉及氫氧化鈉之萃取液 10 mL 置於試管中，以 0.4 g 的過硫酸銨 (ammonium persulphate) [$(NH_4)_2S_2O_8$] 置於高壓釜中消化分解 (以 103.5 kPa，121℃分別消化 60 min 及 90 min)，以 1 N 氫氧化鈉溶液調整 pH 後，以鉬藍法測磷，則分別為碳酸氫鈉和氫氧化鈉萃取全磷。將此濃度分別扣除碳酸氫鈉與氫氧化鈉萃取無機磷，則分別得碳酸氫鈉萃取有機磷 ($NaHCO_3$-OP) 與氫氧化鈉萃取有機磷 (NaOH-OP)。(7) 總磷以 Kjeldahl

法分解，以鉬藍法測定 (Bremner & Mulvaney 1982)。

統計分析

所有資料均以 SAS 軟體進行 ANOVA 分析，再以鄧肯氏多變域比較測定 (Duncan's multiple range test)，比較各處理間之差異。施入之磷肥量與土壤總磷濃度間，以及 Bray-1 有效磷、水萃取磷、碳酸氫鈉萃取無機磷、氫氧化鈉萃取無機磷、鹽酸萃取磷、殘餘磷、有機磷等劃分與土壤總磷濃度間之關係，進行線性迴歸分析 (linear regression analysis)。

結果與討論

土壤性質

經過 9 年水旱田輪作後，其土壤 pH 值在 5.26–6.04 之間，以 Chem-N 處理最低，Comp 處理最高 (表 3)。產酸氮肥 (acid-forming N fertilizer) 之連用會造成土壤 pH 值下降 (Lee *et al*. 2004; Kemmitt *et al*. 2006; Tan & Chen 2012)，然而，本研究連用產酸氮肥 9 年 18 作後，與起始之土壤 pH 值 (5.30) 相比，並未呈現土壤 pH 值下降的現象，應是與肥料性質 (如尿素為中性肥料)、土壤的酸鹼緩衝力大及連用的時間仍短有關。

土壤 EC 值均在 0.41–0.52 dS m^{-1} 之間 (表 3)，以未施肥之 CK 處理最低。雖然不同的處理間有顯著差異，但屬於無鹽度之範圍 (Jackson 1958)，顯示本試驗田的施肥量未累積可溶性鹽。

土壤有機碳濃度介於 12.0–13.2 g kg^{-1} 之間 (表 3)。以施用堆肥與泥炭之 4 處理較高，然而此 4 處理間未達統計之顯著差異；CK、Chem-N 與 GM + 1/3 三處理較低，此 3 處理間亦未達統計之顯著差異。土壤總碳濃度之增加直接反應添加至土壤中之有機質的質與量，雖然堆肥比泥炭易分解 (Niklsch & Joergensen 2001)，然而其施用仍能累積土壤有機碳含量。施用化學氮肥之處理，其有機質來源為作物殘體與雜草，就所有施肥處理而言，在 Chem-N 處理下，回歸及施用到土壤的有機質量是較少的，而施入之化學氮肥因促發反應 (priming effect) (Kuzyakov *et al*. 2000) 而增加土壤有機碳的降解，此由 Chem-N 處理之土壤有機碳濃度略低於起始土壤，即可說明此現象。

除氮、磷外其他營養要素之有效性如表 4。Mehlich 3 可萃取鉀濃度在 63.4–95.3 mg kg^{-1} 之間，以 GM + 1/3 N 處理較高、其次為 Comp、Comp + 1/3 N、Comp + 2/3 N 與 Peat + 1/3 N 四處理，而 CK 與 Chem-N 兩處理最低。Mehlich 3 可萃取鈣濃度為 563–1,636 mg kg^{-1}，除 CK 處理外，其餘處理均在施入化學磷肥時同時施入相同量鈣。施用堆肥之 3 處理則因堆肥含有鈣，使土壤的 Mehlich 3 可萃取鈣濃度增加。土壤的 Mehlich 3 可萃取鎂濃度在 111–234 mg kg^{-1} 之間，亦以施用堆肥之 3 處理較高，而 CK、GM + 1/3 N 與 Peat + 1/3 N 三處理較低，顯示不同肥料處理對鎂濃度略有影響。各處理土壤的 Mehlich 3 可萃取銅與

表 3. 土壤基本化學性質 (一)。
Table 3. Selected chemical properties of the soil (1).

Treatment	pH 1 : 1	EC (dS m^{-1})	Organic C (g kg^{-1})	Total N (mg kg^{-1})	Total P (mg kg^{-1})
CK	5.74 bc^{z}	0.41 c	12.0 b	1,336 c	527 c
Chem-N	5.26 e	0.48 ab	12.1 b	1,367 c	665 b
Comp	6.04 a	0.44 bc	13.1 a	1,986 a	1,127 a
Comp + 1/3 N	5.85 ab	0.52 a	13.1 a	2,126 a	1,178 a
Comp + 2/3 N	5.84 ab	0.48 abc	12.9 a	2,035 a	1,109 a
GM + 1/3 N	5.45 de	0.41 bc	12.2 b	1,572 b	667 b
Peat + 1/3 N	5.55 cd	0.48 abc	13.2 a	2,021 a	741 b

^{z} Within columns, means followed by the same letter(s) are not significantly different (P = 0.05) by Duncan's multiple range test.

表 4. 土壤基本化學性質 (二)。

Table 4. Selected chemical properties of the soil (2).

Treatment	Meh-III[z] K (mg kg^{-1})	Meh-III Ca (mg kg^{-1})	Meh-III Mg (mg kg^{-1})	Meh-III Cu (mg kg^{-1})	Meh-III Zn (mg kg^{-1})
CK	63.4 c[y]	563 c	111 c	1.64 a	2.24 c
Chem-N	65.1 c	989 b	182 b	1.65 a	4.75 b
Comp	84.3 b	1,636 a	234 a	1.02 c	8.94 a
Comp + 1/3 N	84.4 b	1,620 a	226 a	1.08 bc	9.65 a
Comp + 2/3 N	78.7 b	1,565 a	226 a	1.13 bc	9.28 a
GM + 1/3 N	95.3 a	648 c	119 c	1.63 a	3.01 c
Peat + 1/3 N	82.8 b	851 b	128 c	0.69 d	2.61 d

[z] Mehlich III extractable.

[y] Within columns, means followed by the same letter(s) are not significantly different (P = 0.05) by Duncan's multiple range test.

鋅濃度有顯著差異，然而，施用堆肥之處理並未因堆肥中之銅濃度高於其他資材而呈現較高的濃度，應是被土壤吸持 (retain) 而無法顯現其有效性；施用堆肥之處理土壤之 Mehlich 3 可萃取鋅濃度則呈現增加現象，顯示鋅在土壤中累積且提高其植物有效性。此結果說明長期施含鋅濃度高的肥料會造成鋅的累積而使其有效性提高。

氮的分析

氮是自然界分佈最廣的元素之一，然存在於土壤中的氮僅占其中一小部分，其中對植物有效者的比例更少，卻為影響植物生長最明顯的養分因子之一。土壤中的氮大部分為有機態，無機態氮一般不超過總氮量的 5% (Warren & Whitehead 1988)。表 3 顯示，在施氮量相等的前提下，施堆肥之 Comp 處理之土壤總氮濃度顯著高於施用化學氮肥的 Chem-N 處理，即因化學氮肥易被植物吸收利用，甚至流失，而來自堆肥的氮，其中不易礦化的部分會在土壤中累積，而後再礦化。此亦可由 Chem-N 處理與 CK 處理土壤中的總氮濃度未達統計之顯著差異得證，顯示在 9 年 18 期作期間所施入的化學氮肥並未在土壤中留存、累積。堆肥與不同量化學氮肥共用，其各處理總氮濃度未達統計之顯著差異，顯示多施的化學氮肥並未在土壤中留存，即所施的化學氮肥並未增加土壤總氮；而 Comp + 1/3 N、GM + 1/3 N、Peat + 1/3 N 三種處理施入的總氮量是相同的，但 Comp + 1/3 N 與 Peat + 1/3 N 處理之土壤總氮濃度顯著高於 GM + 1/3 N，此結果說明綠肥中的氮殘存在土壤中的量較少，而堆肥與泥炭則相近，應是泥炭或堆肥分解相對較為緩慢，而綠肥被快速分解，在短時間內即礦化，其作用與化學氮肥相似。

無機態氮：土壤中無機態氮 (inorganic N) 主要是硝酸態氮和銨態氮，土壤 pH 值、溫度、水分與植被等因子皆會影響礦化作用。雖然不同的處理間無機態氮濃度有顯著差異，但是其值均低，顯示水稻栽培後土壤中的無機態氮被利用殆盡。在相同氮量之施肥處理中，Comp 處理的濃度顯著高於 Chem-N 處理，顯示殘存在土壤中的堆肥可持續礦化而維持土壤無機態氮的濃度。Flowers & Arnold (1983) 及 Chen & Lee (1997) 之研究結果顯示，施用堆肥可提升土壤氮有效性，亦說明其有較大的礦化潛能 (potential)。Peat + 1/3 N 處理的土壤總氮濃度高，但是無機態氮的濃度並未顯示較高，表示其不易礦化。

有機態氮：土壤中有機態氮主要存在於蛋白質、核酸、胺基酸和腐植質等之中，而以腐植質的比例較高。表 5 顯示本研究各處理之有機態氮占總氮之 97.7–98.7%，以施堆肥與泥炭之四處理顯著高於 CK、Chem-N 與綠肥之處理，綠肥之處理則顯著高於 CK 與 Chem-N 兩處理。泥炭不易分解，施用後會在土壤中逐漸累積碳與氮 (Eklind & Kirchmann 2000)。適量施用堆肥亦可累積土壤有機態氮，經 9 年

表 5. 土壤之無機態氮、有機態氮、可水解全氮與不可水解氮濃度。

Table 5. The concentrations of inorganic nitrogen, organic nitrogen, total hydrolysable nitrogen and non-hydrolysable nitrogen.

Treatment	Inorganic N (mg kg^{-1})	Organic N (mg kg^{-1})	THN[z] (mg kg^{-1})	Nonhydro. N (mg kg^{-1})
CK	22 d[y] (1.6)[x]	1,314 c (98.4)	782 e (58.6)	532 b (39.8)
Chem-N	26 cd (1.9)	1,341 c (98.1)	895 d (65.5)	446 b (32.6)
Comp	46 a (2.3)	1,939 a (97.7)	1,105 bc (55.7)	834 a (42.0)
Comp + 1/3 N	28 bcd (1.3)	2,099 a (98.7)	1,230 a (57.8)	869 a (40.9)
Comp + 2/3 N	40 ab (1.9)	1,996 a (98.1)	1,179 ab (57.9)	817 a (40.1)
GM + 1/3 N	36 abc (2.3)	1,537 b (97.7)	1,019 c (64.8)	518 b (33.0)
Peat + 1/3 N	29 bcd (1.3)	2,092 a (98.7)	1,168 ab (55.1)	924 a (43.6)

[z] THN: total hydrolysable N; Nonhydro. N: non-hydrolyzable N.
[y] Within columns, means followed by the same letter(s) are not significantly different ($P = 0.05$) by Duncan's multiple range test.
[x] The number in parenthesis is the ratio of N fraction to total N in percentage.

施用後與泥炭之效果相近 (表 5)。僅施化學氮肥雖因作物產量比未施肥料之處理高而使回歸至土壤中的作物殘體量較多 (資料未示)，然而此兩處理土壤的有機態氮卻未達統計之顯著差異，可歸因於化學氮肥之促發反應 (Kuzyakov *et al*. 2000) 及處理時間仍短。雖然綠肥比堆肥或泥炭易分解，但與 Chem-N 處理比較，仍有累積土壤有機氮的作用。

有機態氮的化學型態影響土壤氮的有效性，許多研究指出，不同耕作和種植系統對土壤有機氮型態有顯著影響 (Allison 1973; Campbell *et al*. 1986)。Asami (1971) 之研究顯示，長期施用化學氮肥或化學氮肥與廄肥共用，會促進礦化作用和生物固定作用，進而影響土壤中不同的有機態氮劃分。

可水解全氮：可水解全氮占總氮的比例 55.1–65.5% (表 5) (平均為 59.3%)，比不可水解氮 (平均為 40.7%) 約多 20%。可水解氮被認為是可礦化氮 (Keeney & Bremner 1964; Ferguson & Gorby 1971)。施用化學氮肥或有機質肥料比不施肥的 CK 處理之土壤的可水解全氮濃度高，而 CK 與 Chem-N 兩處理的有機態氮未達統計之顯著差異，顯示施肥改變土壤的氮組成，施用堆肥則使可水解與不可水解氮均增加，可水解氮占總氮比例略降，施用化學氮肥使可水解氮占總氮的比例增加。Puranik *et al*. (1978) 及 Xu & Wu (1991) 的研究也顯示廄肥可提高可水解氮濃度；Rao & Ghosh (1981) 之研究則顯示，不施肥料會使可水解氮大量耗損 (在 7 年內耗損量 12%)，亦指出可水解氮相對易降解；其他研究同樣顯示可水解氮在耕作下較易被礦化 (Rendig 1951; Keeney & Bremner 1964; Ferguson & Gorby 1971)。只施堆肥及堆肥與化學氮肥共用的 3 種處理，以 Comp + 1/3 N 處理的可水解全氮濃度顯著高於 Comp 處理，而 Comp + 2/3 N 處理的濃度與 Comp 處理雖未達統計之顯著差異，但是 Comp + 2/3 N 處理有較高的傾向，顯示堆肥與化學氮肥共用，可增加土壤可水解氮，同時占總氮的比例也略增。Keeney & Bremner (1964) 及 Bharadwaj & Omanwar (1994) 之研究也顯示，長期化學氮肥與廄肥共用，可增加土壤可水解氮。有機質肥料與化學肥料共用時，土壤可水解氮的增加比單施化學氮肥者多。施用同量氮肥不同有機質之處理則顯示，泥炭雖然比堆肥與綠肥難分解，但其施用卻比堆肥與綠肥處理者增加較多的可水解全氮 (表 5)。本研究亦依據 Bremner (1965a, 1965b, 1965c) 提出以氮化合物的特性劃分土壤氮的方法，將可水解氮再進行劃分如表 6，分析結果顯示，施用堆肥能增加可水解氮劃分之胺基酸態氮與可水解銨態氮濃度。

不可水解氮：不可水解氮占總氮的比例為 32.6–43.6% (表 5) (平均為 40.7%)，說明土壤中有 40% 氮為未知其組成且不瞭解其特性，Chen *et al*. (1977) 的研究同樣顯示土壤中不

表 6. 土壤中不同劃分可水解氮濃度。

Table 6. The concentrations of different fractions of hydrolysable nitrogen.

Treatment	Ammonium-N (mg kg^{-1})	Amino acid-N (mg kg^{-1})	Amino sugar-N (mg kg^{-1})	Unidentified-N (mg kg^{-1})
CK	263 d[z] (19.7)[y]	341 c (25.5)	46 c (3.4)	106 b (7.9)
Chem	279 cd (20.4)	361 c (26.4)	45 c (3.3)	141 b (10.3)
Comp	347 ab (17.4)	403 abc (20.3)	46 c (2.3)	307 a (15.5)
Comp + 1/3 N	379 a (17.8)	496 a (23.3)	61 bc (2.9)	389 a (18.3)
Comp + 2/3 N	382 a (18.8)	441 abc (21.7)	64 b (3.2)	403 a (19.8)
GM + 1/3 N	318 bc (20.2)	372 bc (23.6)	66 b (4.2)	299 a (19.0)
Peat + 1/3 N	363 a (17.1)	472 ab (22.3)	122 a (5.8)	315 a (14.9)

[z] Within columns, means followed by the same letter(s) are not significantly different (P = 0.05) by Duncan's multiple range test.
[y] The number in parenthesis is the ratio to total N in percentage.

可水解氮占總氮之 20–40%。不可水解氮為鍵結強的氮型態，是生物有效性較低的劃分，因其比可水解氮不易礦化 (Keeny & Bremner 1964)，其占總氮的比例會隨土壤有機質被分解的程度增加而上升。表 5 顯示不可水解氮，以施堆肥與泥炭之四處理較高，而此四處理間則未達統計之顯著差異；以 CK、Chem-N 及 GM + 1/3 N 三處理較低，此三處理間亦未達統計之顯著差異。此結果說明施用化學氮肥造成之促發反應，會增加土壤有機態氮的礦化。綠肥與化學氮肥共用之處理 (GM + 1/3 N 處理)，其不可水解氮低於施用相同氮量之其他有機質肥料處理 (Peat + 1/3 N 與 Comp + 1/3 N 處理)，說明來自綠肥中之有機態氮比堆肥與泥炭易降解，綠肥之易分解，亦造成促發反應。雖然在統計上差異不顯著，Peat + 1/3 N 處理之不可水解氮濃度高於 Comp + 1/3 N 處理，顯示在表觀上 (apparently)，施用泥炭會使土壤中不可水解氮增加多於施堆肥者，而與堆肥共用之化學氮肥則不影響土壤中不可水解氮濃度 (表 5)。

磷的分析

總磷：土壤含磷量受母質、成土作用和施肥的影響 (Dean 1949; Smeck 1985)。由於土壤中極大部分的磷以植物無效狀態存在，因此土壤總磷量與可供當季作物吸收利用的磷含量通常無相關性。本研究顯示，土壤經過 9 年耕作之後，土壤總磷濃度 (表 7) 與磷肥施用量呈現極顯著線性相關 (r = 0.988, P < 0.01, n = 7)，顯示磷肥在土壤中累積。未施肥處理之土壤總磷濃度顯著低於施用磷肥之處理，是由於無外加之磷源，而栽培作物仍持續吸收其中的磷。單用堆肥及堆肥與化學氮肥共用的處理 (Comp、Comp + 1/3 N、Comp + 2/3 N)，其總磷濃度顯著高於其他處理者，係因此三處理之磷肥施入量較其他處理多。而 GM + 1/3 N 處理所施入土壤的磷量雖較 Peat + 1/3 N處理多，但是土壤總磷的濃度卻較低，此應是由於綠肥快速分解，而使其中的磷在短時間礦化而被作物利用。

有效磷：土壤 Bray-1 磷以 CK 處理較低，但與 Chem-N 及 Comp 兩處理未達統計之顯著差異 (表 7)。然而，與起始土壤之 Bray-1 磷 (14.8 mg kg^{-1}) 比，CK 處理經過 9 年未施肥而栽培作物，其有效磷並未有大的改變，可能由其他型態的磷轉變而來 (Kapoor *et al*. 1982; Richards *et al*. 1995)；Comp 處理施入的磷比 Chem-N 處理多，但對 Bray-1 磷影響不大。

單用堆肥及堆肥與化學氮肥共用處理之土壤 Bray-1 磷以 Comp + 1/3 N 與 Comp + 2/3 N 兩處理較高，應與其 pH 值略低有關，與 Verma *et al*. (2005) 之結果相同。Reddy *et al*. (1999) 之研究亦顯示廄肥和化學氮肥共用，會使土壤有效磷濃度增加。除 Comp + 1/3 N 外，GM + 1/3 N 與 Peat + 1/3 N 兩處理的 Bray-1 磷濃度未達統計之顯著差異，以 Comp + 1/3 N 處理較高，除了經由堆肥施入的磷總量較多

表 7. 不同處理土壤之水溶性磷、有效性磷與總磷濃度。

Table 7. The concentrations of water soluble phosphorus, available phosphorus and total phosphorus under different treatments.

Treatment	H_2O-P[z] (mg kg^{-1})	Bray-1 P (mg kg^{-1})	Total-P (mg kg^{-1})	Recovery (%)
CK	3.4 c[y] (0.6)[x]	17.8 b	527 c	106
Chem-N	4.0 c (0.6)	30.5 b	665 b	102
Comp	16.8 a (1.6)	37.3 b	1127 a	92
Comp + 1/3 N	16.1 a (1.4)	65.3 a	1178 a	97
Comp + 2/3 N	15.6 a (1.5)	66.2 a	1109 a	95
GM + 1/3 N	5.8 bc (0.8)	31.9 b	667 b	108
Peat + 1/3 N	7.3 b (1.0)	34.4 b	741 b	104

[z] H_2O-P: Water soluble P; Recovery: Recovery of fractionation.
[y] Within columns, means followed by the same letter are not significantly different (P = 0.05) by Duncan's multiple range test.
[x] The number in parenthesis is the ratio of P fraction to total P in percentage.

(2,254 kg P ha^{-1}) 之外，應與有機質之性質有關。堆肥之施用減少磷被固定因而使磷有效性提高 (Chen 1995)，泥炭與綠肥之效應則較小，因 GM + 1/3 N 處理所施入的磷比 Peat + 1/3 N 處理多，但 Bray-1 磷則相近。Singh & Jones (1976) 之研究顯示，有機質增加或減少土壤磷的吸附作用，與有機質之種類、磷濃度及加入之磷含量有關。Bray-1 有效磷與總磷呈顯著線性正相關 (r = 0.856, P < 0.05, n = 7)，顯示土壤總磷增加時，Bray-1 有效磷亦會增加。

水萃取磷：本研究磷劃分之回收率為 92–108% (表 7)。未施肥之 CK 處理之水萃取磷顯著低於 Comp 處理，但與 Chem-N 處理未達統計之顯著差異。Chem-N 處理之水萃取磷濃度與 CK 處理未達統計之顯著差異，即因所施入的水溶性磷易被作物吸收利用或在土壤中易轉變為不溶性的劃分。Comp、Comp + 1/3 N 及 Comp + 2/3 N 三處理之水溶性磷濃度未達統計之顯著差異，顯示其未受化學氮肥施用之影響。施用不同有機質的處理中，以 Comp + 1/3 N 處理顯著高於 Peat + 1/3 N 及 GM + 1/3 N 兩處理，Peat + 1/3 N 及 GM + 1/3 N 兩處理則未達統計之顯著差異，顯示堆肥、綠肥及泥炭等不同有機資材對土壤中水萃取磷濃度之影響不同，有機資材所含的磷含量越多，則有愈多的有機磷可供礦化，對水萃取磷的增加效果也越大 (Dormaar 1972; Singh & Jones 1976)。 由於堆肥所含的磷含量比泥炭及綠肥多，可礦化釋放的有效磷亦多，但 GM + 1/3 N 處理所施入的磷比 Peat + 1/3 N 處理多，卻未顯示相同的結果，顯示有機質的性質對土壤磷的轉變有甚大的影響。

Bray-1 有效磷與水可萃取磷 (r = 0.810, P < 0.05, n = 7) 呈顯著相關，顯示 Bray-1 磷增加時，水可萃取磷亦增加。

碳酸氫鈉萃取無機磷：碳酸氫鈉溶液萃取無機磷 ($NaHCO_3$-IP) 劃分為植物有效性磷之一部分 (Enwezor 1977; Bowman & Cole 1978)，是溶解度大之鈣結合態磷及部分鋁結合態磷 (Stewart *et al*. 1987)。$NaHCO_3$-IP 濃度與占總磷的比例與鹽酸萃取磷 (HCl-P) 及殘餘磷 (residual-P) 相近，為第 2 高之劃分 (表 8)。Chem-N 處理的濃度顯著高於 CK 處理，顯示未施肥而栽培作物會使此劃分的濃度下降，占總磷的比例也下降。Reddy *et al*. (1999) 亦有相同的研究結果，此亦說明施肥管理會影響磷的組成分，施用化學磷肥直接增加易變動的無機磷源。Comp 處理所施的磷肥多於 Chem-N 處理，因此 Comp 處理之 $NaHCO_3$-IP 濃度顯著高於 Chem-N 處理，但占總磷的比例則相近，顯示經由有機質肥料施入的磷易形成 $NaHCO_3$-IP。Hountin *et al*. (2000) 的研究顯示，長期施用豬糞廄肥增加土壤中 $NaHCO_3$-IP 濃度，也是因經由廄肥施入較多的磷所致。有機質降解所產生的有機酸，會與磷酸鐵與磷酸鋁化合物中的鐵、鋁鉗合，而使無機磷釋

表 **8.** 不同處理土壤之磷劃分濃度。

Table 8. The concentrations of different fractions of phosphorus (P) under different treatments.

Treatment	$NaHCO_3$-IP[z] (mg kg^{-1})	$NaHCO_3$-OP (mg kg^{-1})	NaOH-IP (mg kg^{-1})	NaOH-OP (mg kg^{-1})	HCl-P (mg kg^{-1})	Residual-P (mg kg^{-1})
CK	75 c[y] (13.4)[x]	28 b (5.1)	130 c (23.4)	56 c (10.0)	119 d (21.4)	145 b (26.1)
Chem-N	129 b (19.0)	31 b (4.5)	203 b (29.9)	58 bc (8.5)	112 d (16.5)	142 b (21.0)
Comp	213 a (20.5)	43 ab (4.2)	347 a (33.4)	67 abc (6.4)	199 ab (19.2)	152 b (14.6)
Comp + 1/3 N	226 a (19.7)	41 ab (3.6)	385 a (33.6)	73 ab (6.4)	218 ab (19.0)	187 a (16.3)
Comp + 2/3 N	208 a (19.8)	55 a (5.2)	377 a (35.8)	69 abc (6.5)	178 bc (16.9)	151 b (14.4)
GM + 1/3 N	126 b (17.5)	40 ab (5.5)	207 b (28.8)	78 a (10.8)	113 d (15.8)	149 b (20.7)
Peat + 1/3 N	149 b (19.5)	29 b (3.8)	241 b (31.4)	68 abc (8.8)	136 cd (17.7)	137 b (17.9)

[z] $NaHCO_3$-IP: $NaHCO_3$ extractable inorganic P; $NaHCO_3$-OP: $NaHCO_3$ extractable organic P; NaOH-IP: NaOH extractable inorganic P; NaOH-OP: NaOH extractable inorganic P; HCl-P: HCl extractable inorganic P.
[y] Within columns, means followed by the same letter(s) are not significantly different (P = 0.05) by Duncan's multiple range test.
[x] The number in parenthesis is the ratio of P fraction to total P in percentage.

出，此亦可能是堆肥處理之 $NaHCO_3$-IP 濃度高的原因之一。單用堆肥及堆肥與化學氮肥共用的 3 種處理，所施入的磷肥量相同，其 $NaHCO_3$-IP 濃度與占總磷的比例亦未達統計之顯著差異，顯示所施用之化學氮肥未影響此劃分磷濃度與占總磷之比例 (表 8)。施用不同有機質肥料但相同量氮之 Comp + 1/3 N、Peat + 1/3 N 及 GM + 1/3 N 三處理中，Comp + 1/3 N 處理之 $NaHCO_3$-IP 顯著較高，此與處理施入的磷較多有關外，有機質的性質亦會影響其含量，如 Peat + 1/3 N 及 GM + 1/3 N 兩處理之 $NaHCO_3$-IP 濃度未達統計之顯著差異，但 GM + 1/3 N 處理所施入之磷較 Peat + 1/3 N 處理多。$NaHCO_3$-IP 濃度與 Bray-1 磷濃度 (r = 0.859, P < 0.05, n = 7) 呈顯著線性相關。

氫氧化鈉萃取無機磷：氫氧化鈉萃取無機磷 (NaOH-IP) 主要是土壤中鐵和鋁結合的磷，平均占總磷的比例為 30.9%，除了 CK 處理外，NaOH-IP 在所有處理中為最大之磷劃分 (表 8)。NaOH-IP 為無法立即脫附的磷，為中度不穩定磷 (moderately labile P) (Stewart *et al*. 1987)。Chem 與 Comp 兩處理，其 NaOH-IP 濃度顯著高於未施肥之對照組 (CK)，可歸因於留存肥料磷有甚大的比例轉為此劃分，亦說明磷肥添加會直接增加 NaOH-IP (McKenzie *et al*. 1992; Zhang *et al*. 2004)，未施肥而栽培作物則使此劃分減少。單用堆肥及堆肥與化學氮肥共用的 3 種處理，其 NaOH-IP 濃度未達統計之顯著差異，但是多施化學氮肥之處理有較高的趨勢，顯示化學氮肥會影響堆肥處理土壤中此劃分磷的變化。Comp + 1/3 N、GM + 1/3 N 及 Peat + 1/3 N 三處理中，以 Comp + 1/3 N 處理之 NaOH-IP 濃度較高，可歸因於施用的磷較多，GM + 1/3 N 及 Peat + 1/3 N 兩處理間之 NaOH-IP 濃度未達統計之顯著差異，但以 Peat + 1/3 N 處理略高，而 Peat + 1/3 N 處理之磷施入量少於 GM + 1/3 N 處理，顯示土壤有機質之性質影響磷在土壤中的反應。NaOH-IP 濃度與 Bray-1 磷濃度 (r = 0.907, P < 0.01, n = 7) 呈顯著線性相關。

鹽酸萃取磷劃分：鹽酸溶液萃取磷 (HCl-P) 是土壤中穩定的磷，是生物無效的鈣鍵結磷 (Williams *et al*. 1980)，通常當土壤 pH 值越高或在石灰質土壤，土壤便含有較多的鈣結合磷。Chem-N 及 CK 兩處理之 HCl-P 濃度未達統計之顯著差異，顯示即使施用之磷肥中鈣含量高，以作物需求量之化學磷肥之施用，對此劃分之影響仍小，或許需要經更長的時間之後，才會有顯著差異。Zhang *et al*. (2004) 之研究亦顯示，中度不穩定的磷劃分轉為較穩定的組成分是一緩慢之反應。Reddy *et al*. (1999) 及 Lee *et al*. (2004) 之研究也顯示，長期之作物系統與施肥管理對土壤中穩定的磷劃分影響較小。

單施堆肥及堆肥與化學氮肥共用的 3 種處理的 HCl-P 未達統計之顯著差異，化學氮肥施

用量亦無一致的影響。Comp + 1/3 N、GM + 1/3 N 及 Peat + 1/3 N 三處理中，以 Comp + 1/3 N 處理之 HCl-P 濃度較高，可歸因於施用的磷含量較多，GM + 1/3 N 及 Peat + 1/3 N 兩處理之 HCl-P 濃度未達統計之顯著差異，但仍以 Peat + 1/3 N 處理略高。而 Peat + 1/3 N 處理之磷施入量少於 GM + 1/3 N 處理，亦說明土壤有機質之性質影響磷在土壤中的反應。

殘餘磷：殘餘磷劃分為所有抽出劑抽出後殘存之部分，為不能萃取的二三氧化物 (sesquioxide) 包裹之鐵鋁無機磷、礦物中與鈣鍵結的無機磷，以及較穩定的有機磷，可利用強酸高溫分解而得 (Bauwin & Tyner 1957)，其對植物有效性低 (Chang & Jackson 1957)。Oberson *et al*. (1993) 則認為殘餘磷大部分為有機，此劃分受有機質肥料施用影響。於本研究中並未顯示此項結果，因施用堆肥之 3 種處理，其殘餘磷濃度並不一致。所有處理之殘餘磷中，以對照組所占比例最高，其餘處理則因施肥管理而有不同的變化，主要是因施肥管理影響土壤中殘餘磷外之其他磷劃分的轉變。Reddy *et al*. (1999) 之研究亦顯示施用磷肥不影響殘餘磷量，但 Halvorson & Black (1985) 之研究則顯示定規施肥 (regular fertilization) 之殘存肥料磷可大幅增加不溶與化學穩定的殘餘磷劃分，而後再釋出被利用。在所有處理中，僅 Comp + 1/3 N 處理之殘餘磷濃度顯著較高，但其原因不易解釋，其他處理間則未達統計之顯著差異。

有機磷：土壤的碳酸氫鈉萃取有機磷 ($NaHCO_3$-OP) 及氫氧化鈉萃取有機磷 (NaOH-OP) 濃度是最低的兩個劃分，NaOH-OP 濃度則高於 $NaHCO_3$-OP，即由於氫氧化鈉溶液為較強的試劑，提取較多的 NaOH-OP 所致。Stewart *et al*. (1987) 認為 NaOH-OP 主要來自腐植化合物，是由植物殘體轉變而來。雖然不同處理間的 $NaHCO_3$-OP 有顯著差異，Comp + 2/3 N 處理仍較高，其餘則相近。NaOH-OP 以未施肥濃度較低，以 GM + 1/3 N 處理較高。從分析結果顯示，除施肥管理外，亦有其他因子影響土壤中磷的轉變，或者需要更長的時間，才能顯現其對有機磷的影響。

結論

水旱田輪作下經 9 年 18 作之栽培與施肥管理顯示，化學肥料施用對土壤總氮與有機態氮無增加之效應，但使可水解氮濃度與占總氮的比例上升；堆肥與泥炭增加土壤總氮、有機態氮、可水解氮與不可水解氮；與堆肥共用而多施之化學氮肥並無增加土壤總氮與有機態氮之效用；綠肥可稍提高土壤總氮與可水解氮；堆肥增加胺基酸態氮與可水解銨態氮濃度，施肥管理對於不可辨認的可水解氮與胺基醣態氮濃度之影響小。施用磷肥使磷劃分分佈趨勢改變，留存之肥料磷主要轉變成碳酸氫鈉萃取無機磷與氫氧化鈉萃取無機磷劃分，磷肥施用量多時，此兩劃分相對也增加較多，但有機質之性質會改變磷肥施用量之影響，殘餘磷與鹽酸萃取磷受施肥管理之影響小。綜言之，施用化學肥料並未造成土壤特性的劣化，但對土壤肥力的提高相當有限；施用綠肥則可稍提高土壤總氮與可水解氮 (即有效性氮)；而施用堆肥與泥炭均可顯著提高土壤總氮與可水解氮，但因泥炭為進口資材，價格偏高，且含磷量低。因此，在相同施氮量下，其施磷量相對較少，土壤總磷與有效磷相對較低。就施用堆肥而言，除可顯著增加土壤有機質、提升土壤總氮與總磷含量外，在有效性方面，施用堆肥之處理，其有效性氮與有效性磷含量亦高於其他處理組合，顯示施用堆肥對於增加土壤有效性氮、磷有正面助益，若欲維持較高的土壤肥力建議可施用堆肥。

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引用文獻

Agebenin, J. O. and H. Tiessen. 1994. The effects of soil properties on the different phosphate sorption by semiarid soils from Northeast Brazil. Soil Sci. 157:36–45.

Allen, A. L., F. J. Stevenson, and L. T. Kurtz. 1973. Chemical distribution of fertilizer nitrogen in soil as revealed by nitrogen-15 studies. J. Environ. Qual. 2:120–124.

Allison, F. E. 1973. Soil organic matter and its role in crop production. p.58–66. *in*: Development in Soil Science. (Allison, F. E., ed.) Elsevier. Amsterdam, The Netherlands. 634 pp.

Asami, T. 1971. Immobilization and mineralization of nitrogen compounds in paddy soils. V. Distribution of immobilized nitrogen to various organic N fractions. J. Sci. Soil Manure 42:103–108.

Bauer, A. and A. L. Black. 1994. Quantification of the effect of soil organic matter content on soil productivity. Soil Sci. Soc. Amer. J. 58:185–193.

Bauwin, G. R. and E. H. Tyner. 1957. The nature of reductant soluble phosphorus in soils and concretions. Soil Sci. Soc. Amer. Proc. 21:250–257.

Bharadwaj, V. and P. K. Omanwar. 1994. Long-term effects of continuous rotational cropping and fertilization on crop yields and soil properties. II. Effects on EC, pH, organic matter and available nutrients of soil. J. Indian Soc. Soil Sci. 42:387–392.

Bowman, R. A. and C. V. Cole. 1978. An exploratory method for fractionation of organic phosphorus from grassland soil. Soil Sci. 125:95–101.

Bremner, J. M. 1965a. Inorganic forms of nitrogen. p.1179–1237. *in*: Methods of Soil Analysis Part 2. (Black, C. A., ed.) American Society of Agronomy Inc. Madison, WI. 1572 pp.

Bremner, J. M. 1965b. Organic forms of nitrogen. p.1238–1255. *in*: Methods of Soil Analysis Part 2. (Black, C. A., ed.) American Society of Agronomy Inc. Madison, WI. 1572 pp.

Bremner, J. M. 1965c. Total nitrogen. p.1149–1178. *in*: Methods of Soil Analysis Part 2. (Black, C. A., ed.) American Society of Agronomy Inc. Madison, WI. 1572 pp.

Bremner, J. M. and C. S. Mulvaney. 1982. Salicylic acid-thiosulfate modification on Kjeldahl method to include nitrate and nitrite. p.621–622. *in*: Methods of Soil Analysis Part 2. Chemical and Microbiological Properties. 2nd ed. (Page, A. L., ed.) American Society of Agronomy: Soil Science Society of America. Madison, WI. 1692 pp.

Campbell, C. A., V. O. Biederbeck, F. Selles, M. Schnitzer, and J. W. Stewart. 1986. Effect of manure and P fertilizer on properties of a black chernozem in Southern Saskatchewan. Can. J. Soil Sci. 66:601–614.

Chang, S. C. and M. L. Jackson. 1957. Fractionation of soil phosphorus. Soil Sci. 84:133–144.

Chen, C. 1971. Study on the results of experiments on fertility of paddy soils in regions north of Miaoli in Taiwan. J. Agric. Res. China 20:21–39. (in Chinese with English abstract)

Chen, J. H. 1995. Effect of organicfertilizers addition on the availability and mineralization of soil phosphorus. J. Chinese Agric. Chem. Soc. 33:533–549. (in Chinese with English abstract)

Chen, J. H. and J. Y. Lee. 1997. The effects and evaluation of composts addition on the availability and mineralization of nitrogen in strongly acid soil. J. Agric. Assoc. China New Ser. 179:73–93. (in Chinese with English abstract)

Chen, Y., F. J. Sowden, and M. Schnitzer. 1977. Nitrogen in Mediterranean soils. Agrochimica 27:7–14.

Darwish, O. H., N. Persaud, and D. C. Martens. 1995. Effect of long-term application of animal manure on physical properties of three soils. Plant Soil 176:289–295.

Dean, L. A. 1949. Fixation of soil phosphorus. Adv. Agron. 1:391–411.

Doran, J. W., M. Sarrantino, and M. A. Liebig. 1996. Soil health and sustainability. Adv. Agron. 56:1–54.

Dormaar, J. F. 1972. Seasonal patterns of soil organic phosphorus. Can. J. Soil Sci. 52:107–122.

Eghball, B., B. J. Wienhold, J. E. Gilley, and R. A. Eigenberg. 2002. Mineralization of manure nutrients. J. Soil Water Conserv. 57:470–473.

Eklind, Y. and H. Kirchmann. 2000. Composting and storage of organic household waste with different litter amendments. II: Nitrogen turnover and losses. Bioresour. Technol. 74:125–133.

Enwezor, W. O. 1977. Soil testing for phosphorus in some Nigerian soils: I. Comparison of methods of determining available phosphorus in soils of southeastern Nigeria. Soil Sci. 123:48–53.

Ferguson, W. S. and E. J. Gorby. 1971. Effects of various periods of seed down to alfalfa and brome grass on soil nitrogen. Can. J. Soil Sci. 51:65–73.

Fließbach, A., H. Oberholzer, L. Gunst, and P. Mäder. 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric. Ecosyst. Environ. 118:273–284.

Flowers, T. H. and P. W. Arnold. 1983. Immobilization and mineralization of nitrogen in soil incubated with pig slurry or ammonium sulphate. Soil Biol. Biochem. 15:329–335.

Hadas, A. and R. Pornoy. 1994. Nitrogen and carbon mineralization rates of composted manures incubated in soils. J. Environ. Qual. 23:1184–1189.

Halvorson, A. D. and A. L. Black. 1985. Fertilizer phosphorus recovery after seventeen years of dryland cropping. Soil Sci. Soc. Amer. J. 49:933–937.

Hedley, M. J., J. W. B. Stewart, and B. S. Chauhan. 1982. Changes in the inorganic and organic soil phospho-

rus fractions induced by cultivation practices and by laboratory incubation. Soil Sci. Soc. Amer. J. 46:970–976.

Hountin, J. A., A. Karam, D. Couillard, and M. P. Cescas. 2000. Use of a fraction procedure to assess the potential for P movement in a soil profile after 14 years of liquid pig manure fertilization. Agric. Ecosyst. Environ. 78:77–84.

Jackson, M. L. 1958. Soluble salt analysis for soils and waters. p.227–271. *in*: Soil Chemical Analysis. (Jackson, M. L., ed.) Prentice-Hall. Englewood Cliffs, NJ. 485 pp.

Kapoor, M. L., G. S. Sekhon, and B. Singh. 1982. Nitrogen, phosphorus and potassium nutrition of maize and wheat from the surface and subsurface layers. Indian J. Ecol. 9:264–269.

Kaur, K., K. K. Kapoor, and A. P. Gupta. 2005. Impact of organic manures with and without mineral fertilizers on soil chemical and biological properties under tropical conditions. J. Plant Nutr. Soil Sci. 168:117–122.

Keeney, D. R. and J. M. Bremner. 1964. Effect of cultivation on the nitrogen distribution in soils. Soil Sci. Soc. Amer. Proc. 28:653–656.

Kelley, K. R. and E. J. Stevenson. 1995. Forms and nature of organic N in soil. Fert. Res. 42:1–11.

Kemmitt, S. J., D. Wright, K. W. T. Goulding, and D. L. Jones. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biol. Biochem. 38:898–911.

Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32:1485–1498.

Lee, C. H., C. Y. Park, K. D. Park, W. T. Jeon, and P. J. Kim. 2004. Long-term effects of fertilization on the forms and availability of soil phosphorus in rice paddy. Chemosphere 56:299–304.

Lee, T. Z. 1971. Effects of $CaCO_3$, $CaSiO_3$, and organic manure on the growth and yield of rice on a flooded acid latosolic soil. J. Agric. Res. China 20:47–55. (in Chinese with English abstract)

Lee, Y. and S. Lian. 2004. Contents and variations of the major constituents in the organic manures in Taiwan. J. Agric. Res. China 46:181–189. (in Chinese with English abstract)

Lian, S., C. H. Wang, and S. C. Lee. 1996. Tracing of the labelled urea-N flood-fertigated with different depths of water at knee-high stage under no-till conditions. J. Agric. Res. China 45:47–56. (in Chinese with English abstract)

Lin, C. F., A. H. Chang, and C. C. Tseng. 1973a. The nitrogen status and nitrogen supplying power of Taiwan Soils. J. Agric. Res. China 22:186–203. (in Chinese with English abstract)

Lin, C. F., T. S. Lee, A. H. Chang, and C. Y. Cheng. 1973b. Effects of some long-term fertilizer treatments on the chemical properties of soil and yield of rice. J. Agric. Res. China 22:241–262. (in Chinese with English abstract)

Lin, C. F., W. K. Wu, and A. H. Chang. 1977. Effects of soil composition on the relationships between P-uptake of rice plant and soil test values for phosphorus fertilizer requirements. J. Agric. Res. China 26:138–146. (in Chinese with English abstract)

Lin, Y. W., T. S. Liu, and C. H. Wang. 2003. Study on nitrogen mineralization characteristics of organic materials. J. Agric. Res. China 52:178–190. (in Chinese with English abstract)

Masto, R. E., P. K. Chhonkar, D. Singh, and A. K. Patra. 2006. Changes in soil biological and biochemical characteristics in a long-term field trial on a subtropical Inceptisol. Soil Biol. Biochem. 38:1577–1582.

McKenzie, R. H., J. W. B. Stewart, J. F. Dormaar, and G. B. Schaalje. 1992. Long-term crop rotation and fertilizer effects on phosphorus transformations. I. In a Chernozemic soil. Can. J. Soil Sci. 72:569–579.

McLean, E. O. 1982. Soil pH and lime requirement, p.199–224. *in*: Methods of Soil Analysis Part 2 Chemical and Microbiological Properties. 2nd ed. (Page, A. L., ed.) American Society of Agronomy: Soil Science Society of America. Madison, WI. 1142 pp.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15:1409–1416.

Murphy, J. and J. P. Riley. 1962. A modified single solution for determination of phosphate in natural waters. Anal. Chim. Acta 27:31–36.

Niklsch, H. and R. G. Joergensen. 2001. Decomposition of peat, biogenic municipal waste compost, and shrub/grass compost applied in different rates to a silt loam. J. Plant Nutr. Soil Sci. 164:365–369.

Oberson, A., J. C. Fardeau, J. M. Besson, and H. Sticher. 1993. Soil phosphorus dynamics in cropping systems managed according to conventional and biological agricultural methods. Biol. Fertil. Soils 16:111–117.

Olk, D. C., K. G. Cassman, E. W. Randall, P. Kinchesh, L. J. Sanger, and J. M. Anderson. 1996. Changes in chemical properties of organic matter with intensified rice cropping in tropical lowland soil. Eur. J. Soil Sci. 47:293–303.

Olsen, S. R. and L. E. Sommers. 1982. Phosphorus. p.403–430. *in*: Methods of Soil Analysis Part 2. Chemical

and Microbiological Properties. 2nd ed. (Page, A. L., ed.) American Society of Agronomy: Soil Science Society of America. Madison, WI. 1142 pp.

Puranik, R. B., D. K. Ballal, and N. K. Barde. 1978. Studies on nitrogen forms as affected by long term manuring and fertilization in vertisols. J. Indian Soc. Soil Sci. 26:169–172.

Rao, A. S. and A. B. Ghosh. 1981. Effect of continuous cropping and fertilizer use on the organic nitrogen fractions in a typic Ustochrept soil. Plant Soil 62:377–383.

Rasmussen, P. E. and H. P. Collins. 1991. Long-term impacts of tillage, fertilizer, and crop residue on soil organic matter in temperate semiarid regions. Adv. Agron. 45:93–134.

Reddy, D. D., A. S. Rao, and P. N. Takkar. 1999. Effects of repeated manure and fertilizer phosphorus additions on soil phosphorus dynamics under a soybean wheat rotation. Biol. Fertil. Soils 28:150–155.

Reeves, D. W. 1997. The role of soil organic matter in maintaining soil quality in continuous cropping systems. Soil Till. Res. 43:131–167.

Rendig, V. V. 1951. Fractionation of soil nitrogen and factors affecting the distribution. Soil Sci. 71:253–268.

Rhoades, J. D. 1982. Soluble salts. p.167–178. *in*: Methods of Soil Analysis Part 2. Chemical and Microbiological Properties. 2nd ed. (Page, A. L., ed.) American Society of Agronomy: Soil Science Society of America. Madison, WI. 1142 pp.

Richards, J. E., T. E. Bates, and S. C. Sheppard. 1995. Changes in the forms and distribution of soil phosphorus due to long-term corn production. Can. J. Soil Sci. 75:311–318.

Schmidt, J. P., S. W. Buol, and E. J. Kamprath. 1996. Soil phosphorus dynamics during seventeen years of continuous cultivation: fractionation analyses. Soil Sci. Soc. Amer. J. 60:1168–1172.

Singh, B. B. and J. P. Jones. 1976. Phosphorus sorption and desorption characteristics as affected by organic residues. Soil Sci. Soc. Amer. J. 40:389–394.

Smeck, N. E. 1985. Phosphorus dynamics in soils and landscapes. Geoderma 36:185–199.

Stewart, J. W. B., I. P. O'Halloran, and R. G. Kachanoski. 1987. Influence of texture and management practices on the forms and distribution of soil phosphorus. Can. J. Soil Sci. 67:147–163.

Sui, Y., M. L. Thompson, and C. Shang. 1999. Fractionation of phosphorus in a mollisol amended with biosolids. Soil Sci. Soc. Amer. J. 63:1174–1180.

Tan, T. W. and K. N. Chen. 2012. Effect of long-term application of chemical fertilizers on soil pH in different cropping systems. J. Agric. Res. China 61:355–360. (in Chinese with English abstract)

Tiessin, H., J. W. B. Stewart, and C. V. Cole. 1984. Pathways of phosphorus transformations in soils of offering pedogenesis. Soil Sci. Soc. Amer. J. 48:853–858.

Tseng, H. D. and C. H. Wang. 1959. A study on the methods of determining available phosphorus of paddy soils in Taiwan. J. Agric. Res. China 8:11–20. (in Chinese with English abstract)

Tseng, Y. I. and C. H. Wang. 1967. Study on the behavior of urea in soils. J. Agric. Res. China 16:58–68. (in Chinese with English abstract)

Verma, S., S. K. Subehia, and S. P. Sharma. 2005. Phosphorus fractions in an acid soil continuously fertilized with mineral and organic fertilizers. Biol. Fertil. Soils 41:295–300.

Warren, G. P. and D. C. Whitehead. 1988. Available soil nitrogen in relation to fractions of soil nitrogen and other soil properties. Plant Soil 112:155–165.

Williams, J. D. H., J. K. Syers, and T. W. Walker. 1967. Fractionation of soil inorganic phosphate by a modification of Chang and Jackson's Procedure. Soil Sci. Soc. Amer. Proc. 31:736–739.

Williams, J. D. H., T. Mayer, and J. O. Nriagu. 1980. Extractability of phosphorus from phosphate minerals common in soils and sediments. Soil Sci. Soc. Amer. J. 44:462–465.

Xu, C. X. and S. R. Wu. 1991. Composition of organic nitrogen in Lou soil and its changes under fertilization conditions. Chinese J. Soil Sci. 27:54–56. (in Chinese with English abstract)

Xu, Y. C., Q. R. Shen, and W. Ran. 2003. Content and distribution of forms of organic N in soil and particle size fractions after long-term fertilization. Chemosphere 50:739–745.

Zhang, T. Q., A. F. MacKenzie, B. C. Liang, and C. F. Drury. 2004. Soil test phosphorus and phosphorus fractions with long-term phosphorus addition and depletion. Soil Sci. Soc. Amer. J. 68:519–528.

Effect of Different Fertilization Managements on the Changes of Soil Nitrogen and Phosphorus Fractions under Rice and Corn Rotation for Nine Years

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Abstract

Chou, E. T., C. H. Wang, R. S. Chung, and C. L. Chen. 2016. Effect of different fertilization managements on the changes of soil nitrogen and phosphorus fractions under rice and corn rotation for nine years. J. Taiwan Agric. Res. 65(3):313–327.

Nitrogen (N) and phosphorus (P) are the most common limiting nutrient on the growth and yield of crops. There are many factors that influence the availability and transformation of N and P in soils. The objectives of this study were to evaluate the effects of different fertilization managements and application of different kinds of fertilizers on the different fractions of soil N and P under upland-lowland (corn and rice) rotation for nine years. There were seven treatments, namely, 1. CK (without fertilization), 2. Chem-N (applying chemical N fertilizer only), 3. Comp (applying compost with the same rate as N of Chem-N treatment), 4. Comp + l/3 N (applying compost complemented with 33% of chemical N fertilizer as Chem-N treatment), 5. Comp + 2/3 N (applying compost complemented with 66% of chemical N fertilizer as Chem-N treatment), 6. GM + 1/3 N (applying green manure complemented with 33% of chemical N fertilizer as Chem-N treatment), and 7. Peat + 1/3 N (applying peat complemented with 33% of chemical N fertilizer as Chem-N treatment). The methods proposed by Bremner (1965a, 1965b, 1965c) and Sui *et al*. (1999) were used to fractionate N and P, respectively, into different fractions. The results indicated that soil total N and organic N were not affected the proportion of hydrolysable N increased by the application of chemical N fertilizer. Application of compost and peat resulted in increased soil total N and organic N. Furthermore, both hydrolysable and nonhydrolyzable N increased. The chemical N fertilizer applied with compost had no effect on the concentrations of soil total N and organic N. Application of green manure gradually increased soil total N and hydrolysable N. The unidentified hydrolysable N and amino sugar-N were less affected by different fertilization managements. Compost increased soil amino acid-N and hydrolysable ammonium N. Different fertilization managements resulted in change of concentration and proportion of different soil P fractions. Residual fertilizer P predominantly became sodium bicarbonate extractable inorganic P ($NaHCO_3$-IP) and sodium hydroxide extractable inorganic P (NaOH-IP). The greater the P fertilizer applied, the more the increase in these fractions of P. The characteristics of organic fertilizer affected the transformation of residual fertilizer P in the soil. Residual P and hydrochloric acid extractable P were less affected by fertilization management.

Key words: Hydrolyzable nitrogen, Nonhydrolyzable nitrogen, Sodium bicarbonate extractable phosphorus, Hydrochloric acid extractable phosphorus.

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研究報告

利用分子標幟輔助導入 *Saltol* QTL 提升水稻「台稉九號」之耐鹽性

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摘要

溫甯翔、郭介煒、郭素真、林彥蓉、吳永培。2016。利用分子標幟輔助導入 *Saltol* QTL 提升水稻「台稉九號」之耐鹽性。台灣農業研究 65(3):328–347。

本研究利用台灣優質稉稻品種「台稉九號」(‘TK9’) 為輪迴親，耐鹽秈稻品種 ‘Pokkali’ 為貢獻親，利用分子標幟輔助回交法將 ‘Pokkali’ 之 *Saltol* QTL 導入 ‘TK9’ 中。過程中各回交世代均先進行 *Saltol* QTL 之前景選拔，獲選後代再經分子標幟輔助之背景選拔。結果發現帶 *Saltol* QTL 對偶基因之回交子代，其 BC_1F_1、BC_2F_1 及 BC_2F_2 世代獲選植株遺傳背景回復至輪迴親比率平均分別為 71.5、85.4 及 82.7%。與傳統回交育種 BC2F1 理論值 87.5% 相較，遺傳背景回復率有稍低的現象，顯示兩親本間可能遺傳背景較遠或發生遺傳拖曳之現象。又 ‘Pokkali’、CWY981126、‘IR64’ 及 10 個 BC_2F_3 品系於五葉齡期分別以 100、150、200、250 及 300 mM NaCl 溶液進行鹽分逆境處理，結果 ‘Pokkali’ 在 100、150 及 200 mM NaCl 等鹽分濃度下均有高的耐鹽性表現，而 BC_2F_3 回交後代則以 STK7 及 STK8 等 2 個品系耐鹽性表現較佳。而相同材料分別於五葉齡及最高分蘗期以 200 mM NaCl 逆境進行不同生長時期耐鹽性評估，其中秧苗五葉齡鹽分逆境處理下，以品系 STK8 的耐鹽等級表現最佳，而 STK4 及 STK7 亦有不錯的耐鹽性表現；至於成株期之最高分蘗期則以 STK7 及 STK8 的耐鹽性表現最佳，由回交品系 BC_2F_3 耐鹽表現，顯示本試驗已順利將 *Saltol* QTL 導入 ‘TK9’ 中，且成功培育具優良耐鹽性之 BC_2F_3 回交品系，未來可供國內鹽化土壤栽培利用。

關鍵詞：分子標幟輔助選拔、耐鹽、水稻。

前言

水稻 (*Oryza sativa* L.) 是全世界重要的糧食作物之一，生長發育與產量受不同生物與非生物逆境影響甚鉅。鹽分逆境是屬於非生物逆境的一種，主要影響全世界灌溉與旱作農業，全世界作物生產嚴重受鹽害威脅之農業耕地約有 800 百萬公頃，佔全世界總面積約 6% (Munns 2005, FAO 2008)。至 2050 年估計有超過 50% 的農業耕地會面臨鹽分與乾旱逆境 (Wang *et al*. 2003)，伴隨著世界人口數量持續增加，預計在 2050 年將達到 91 億 (Negrão *et al*. 2011)，人口的增加使得一些耕作土地轉變為工業及居住用地，迫使水稻之耕作轉移至生產力低的鹽分地。水稻生產必須增加超過 50% 才可滿足人類對糧食的需求，將使得糧食短缺之現象雪上加霜，故培育耐鹽水稻已成為全球育種的首要目標之一。

土壤中離子濃度過高 (主要為 Cl^- 和 Na^+)，會使植物根部的滲透壓調節功能喪失，造成鹽分逆境。鹽分逆境不僅造成土壤結構的

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改變，使土壤的通氣量和水力傳導降低，又會降低土壤水分潛勢，阻礙水稻對水分及礦物質的吸收，進而抑制稻株的生長與發育。土壤大量的 Na^+ 與 Cl^- 等毒性離子直接被水稻吸收，Na^+ 及 Cl^- 的累積造成體內離子及滲透調節失衡，使氣孔關閉。當 CO_2 同化受限，致使電子傳送反應受阻，進而產生活化氧族 (reactive oxygen species; ROS)，導致細胞受損、光合作用及蛋白質合成受抑制，造成生長勢降低、不正常發育或產量降低，重者則導致植株死亡 (Darwish *et al*. 2009; Tavakkoli *et al*. 2010)。Munns & James (2003) 指出 Na^+ 累積在地上部與鹽分逆境下水稻植株的存活率有相關性。因此，對於克服鹽分逆境有效的方法，即為維持植株中低 Na^+ 濃度。也有研究指出，K^+ 對於植株耐鹽性也有所影響。維持地上部較低的 Na^+/K^+ 比率，對耐鹽性的強度至關重要 (Ren *et al*. 2005; Yamaguchi & Blumwald 2005)。據此研究結果，作物的耐鹽表現可以透過 Na^+ 的排除與隔離、K^+ 吸收及維持 Na^+/K^+ 比率來提高。

國際水稻基因體計畫 (International Rice Genome Sequencing Project; IRGSP) 完成稉稻「日本晴」(‘Nipponbare’) 全基因體共約 389 Mb 的解序 (IRGSP 2005)，促進了各種分子標幟的發展。隨後應用在水稻育種上，不僅可在早世代進行候選植株的選拔，更可縮短水稻育種的年限，提高了育種效率和精確性 (Collard & Mackill 2008)，此即為分子標幟輔助選拔 (marker-assisted selection; MAS)。MAS 可在不受環境干擾下準確挑選目標植株，並在全生育期進行選拔，應用於幼苗期篩選更可減少田間種植數量、人力和物力等耗費，尤其可將多基因導入或堆疊 (pyramiding) 於同一優良水稻品種中。目前水稻育種上利用 MAS 技術的成果相當多，如：國際稻米研究所將耐淹浸 *Sub1* QTL，導入 ‘Swarna’、‘IR64’ 和 ‘Sumba Mahsuri’ 等水稻品種，除了保有品種原有的高產和優良農藝性狀外，更增加了耐淹之特性 (Neeraja *et al*. 2007)；此外，也有將抗稻熱病 (Jiang *et al*. 2012)、抗白葉枯病 (Pradhan *et al*. 2015)、抗褐飛蝨 (Hu *et al*. 2013)、香味 (Salgotra *et al*. 2012)、穀粒產量 (Swamy & Kumar 2013) 等應用 MAS 進行改良品種的實例，能有效提高水稻產量品質、抗病及抗逆境的特性。Ruengphayak *et al*. (2015) 更以多次回交的方式，將耐淹之 *sub1*、抗稻熱病之 *qBL1* 及 *qBL11*、抗白葉枯病之 xa5 及 Xa21、抗褐飛蝨基因 *sesquiterpene synthase* (*TP*) 及 *qBph3* 和決定支鏈澱粉合成之 *starch synthase IIa* (*SSIIa*) 等基因和基因座，導入到高產的泰國香米品種 ‘PinK3’ 中，透過 4 年 7 次的傳統回交法配合 MAS，不僅改良了品種耐逆境及稻米品質之特性，更能縮短新品種育成時間。

在耐鹽水稻品種育種上，印度已成功培育出 ‘Damodar’、‘Pokkali’、‘Nona Bokra’ 和 ‘Bhura Ratha’ 等耐鹽的水稻品種，但是大部分品種的產量表現均不佳 (Singh & Mishra 2004)。為了提高耐鹽品種在鹽分逆境下的產量表現，Islam & Gregorio (2013) 利用分子標幟輔助回交育種 (marker assisted back-cross; MABC)，將 *Saltol* 導入到印度秈稻品種 ‘BR11’、‘BRRI dhan28’ 和 ‘BRRI dhan29’ 中，不僅提高品種之耐鹽特性，更在鹽分逆境下維持了產量。Gregorio *et al*. (1997) 利用 ‘Pokkali’ 與 ‘IR29’ 進行雜交後自交產生之重組自交系，進一步在水稻第 1 條染色體上 10.7–12.2 Mb 發現 1 個與控制 Na^+/K^+ 比率有關之 QTL，命名為 *Saltol*。Thomson *et al*. (2010) 針對 *Saltol* QTL 進行檢測，顯示 *Saltol* QTL 對耐鹽等級變異的解釋率約為 27%，且對水稻耐鹽性有較大的影響。另外，根據圖譜定位找到了 3 個與幼苗存活天數相關之 QTLs，其幼苗存活天數會因耐鹽 QTLs 堆疊數目的增加而提高植株之耐鹽性 (Cheng *et al*. 2012)。國際稻米研究所利用 ‘Pokkali’ 和 ‘IR29’ 雜交之 F_8 重組自交系族群進行之耐鹽研究中，結果發現 1 個高耐鹽品系 FL478 (IR 66946-3R-178-1-1)，目前已利用 MABC 方式，將 FL478 之 *Saltol* 轉入優良品種中，期成功培育出高耐鹽且高產的新品系 (Thomson *et al*. 2010)；許多水稻耐鹽特性之研究學者於此區間設計多個緊密連鎖之分子標幟，本試驗選用 1 個緊密連鎖之分子標幟 CH0113 及 2 個鄰近兩側之分子標

幟，分別為 RM10695 及 RM10793，用以篩選出帶有 ‘Pokkali’ 之 *Saltol* QTL 基因片段的回交後代。

本試驗利用耐鹽秈稻 ‘Pokkali’ 為貢獻親，透過分子標誌輔助回交育種方式，將 ‘Pokkali’ 提供之耐鹽 *Saltol* QTL 轉移至台灣粳稻品種「台粳九號」(‘TK9’)，過程中每 1 個世代均透過 200 mM 之高鹽分逆境篩選，而存活植株再利用分子標幟進行前景選拔 (foreground selection)，篩選具耐鹽性且再以背景選拔選出與 ‘TK9’ 遺傳背景相似度高之水稻新品系，並藉此建立台灣分子標幟輔助耐鹽水稻的育種模式，因應未來氣候變遷下耐鹽逆境之水稻育種、栽培及糧食生產時需求。

材料與方法

試驗材料

回交族群之建立：本試驗以秈稻品種 (*Oryza sativa* L. indica) ‘Pokkali’ 作為貢獻親，而粳稻品種 (*Oryza sative* L. japonica) ‘TK9’ 則作為輪迴親，‘Pokkali’ 為印度沿海栽培常用的極耐鹽之秈稻品種，所帶有之 *Saltol* QTL 是控制目標性狀耐鹽性的 QTL，因此以其做為貢獻親 (Thomson *et al*. 2010)；而 ‘TK9’ 是台灣優質米推薦之水稻品種，食味品質優良，故在本研究中作為導入 *Saltol* QTL 所需之輪迴親。以 ‘Pokkali’ 及 ‘TK9’ 雜交後，得到 F1 種子，再與輪迴親 ‘TK9’ 重複 2 次回交及自交，分別建立分子標幟輔助選拔所需 BC_1F_1、BC_2F_1、BC_2F_2 及 BC2F3 之各世代材料 (圖 1)。在各回交世代中，BC_1F_1 植株有 268 株、BC_2F_1 為 320 株、而 BC_2F_2 世代則為 608 株，在各世代稻株秧苗五葉齡時先以 200 mM NaCl 溶液進行鹽分逆境處理，其存活植株再進一步進行分子標幟前景選拔，確認帶 *Saltol* QTL 之植株隨後則進行分子標幟背景選拔。其中 BC_1F_1 經分子標幟背景選拔獲選之植株為 10 株，即挑選出與輪迴親 ‘TK9’ 遺傳背景相似度最高之 10 株移至田間種植，供作為母本進行回交。至於 BC_2F_1 及 BC_2F_2 獲選之耐鹽植株同樣進行分子標幟前景及背景選拔，分別選擇

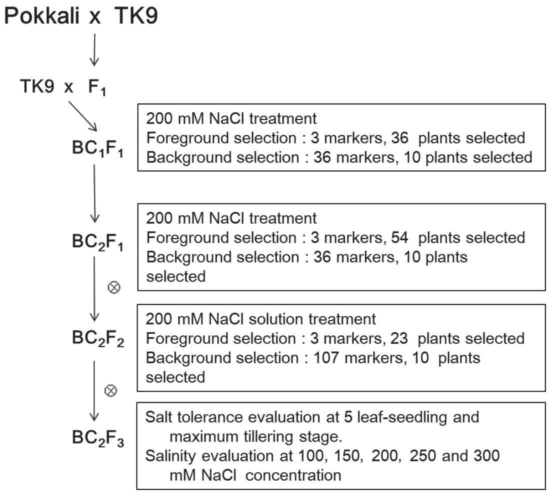


圖 1. 分子標幟輔助 Saltol QTL 導入 ‘TK9’ 之回交育種流程，包含各世代 200 mM NaCl 鹽分處理、前景及背景選拔之分子標幟數量及獲選株數。

Fig. 1. Marker-assisted backcross breeding scheme for introgression *Saltol* QTL into ‘TK9’ with details of 200 mM NaCl solution treatment, markers used for foreground and background selection, and the numbers of plants selected in each generation.

帶有 *Saltol* QTL 且遺傳背景與 ‘TK9’ 相似度最高之 10 單株，獲選單株分別收穫自交 BC_2F_3 種子。

耐鹽表現調查

耐鹽等級調查：水稻種子以培養皿進行消毒及浸種發芽後，點播於 60 cm × 50 cm 之 80 孔的水耕栽培盤進行水耕栽培，每孔 1 株，培養液使用改良式木村氏 B 培養液配方 (Yoshida *et al*. 1976)。待全盤植株平均生長達 5–6 葉齡時，改以含有 200 mM NaCl 之水耕液進行鹽分逆境處理，並進行植株耐鹽等級之調查。調查耐鹽等級依據修改 IRRI 於 2002 年所制定鹽害等級分級標準之水稻標準評估系統 (Standard Evaluation System for Rice; SES) 手冊，其分為 6 個等級，分別為 0 級：植株正常生長；1 級：植株葉片捲曲或下位葉黃化；3 級：植株葉片前端乾枯白化；5 級：植株葉片約 1/2 乾枯白化；7 級：植株葉片約 2/3 乾枯白化；9 級：植株完全死亡 (圖 2)。

耐鹽品系不同發育時期之調查：BC_2F_2 世代利用分子標幟輔助選拔之 10 個單株分別

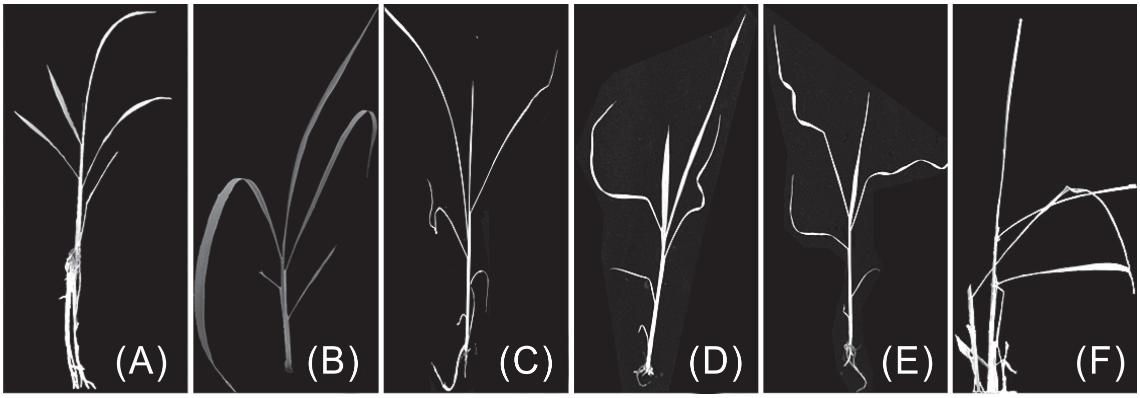


圖 2. 水稻秧苗期耐鹽等級調查標準。(A) 植株生長正常，耐鹽等級為 0；(B) 植株葉片捲曲或下位葉枯黃，耐鹽等級為 1；(C) 植株葉片先端乾枯黃化，耐鹽等級為 3；(D) 植株葉片約 1/2 乾枯黃化，耐鹽等級為 5；(E) 植株葉片約 2/3 乾枯黃化，耐鹽等級為 7；(F) 植株完全乾枯黃化，耐鹽等級為 9。

Fig. 2. Standards used for evaluating salt-tolerance in rice seedling. (A) When plant growth is normal, the scale of salt-tolerance is denoted 0; (B) some leaves are rolled or discolored, the scale of salt-tolerance is denoted 1; (C) some leaf tips are drying, the scale of salt-tolerance is denoted 3; (D) about 1/2 leaves are drying, the scale of salt-tolerance is denoted 5; (E) about 2/3 leaves are drying, the scale of salt-tolerance is denoted 7; (F) and whole plant nearly dead and wilted, the scale of salt-tolerance is denoted 9.

收穫自交種子，並於 BC2F3 世代成立品系，即 STK1、STK2、STK3、STK4、STK6、STK7、STK8、STK9、STK10、STK11 等 10 個品系。10 品系分別於五葉齡及最高分櫱期兩個不同生長階段進行鹽分逆境處理，並以輪迴親本 ‘TK9’ 作為對照品種。五葉齡之試驗利用 34 cm × 27 cm 之 96 孔水耕栽培盤培育幼苗，並於苗齡達五葉齡時添加 200 mM NaCl 水耕液進行鹽分逆境處理。試驗以 RCBD (randomized complete block design; RCBD) 設計排列，2 重複，每重複 8 株。而最高分櫱期試驗則利用口徑 34 cm 之塑膠圓桶並加入 8.5 kg 田土，以自來水灌溉，維持水面為土面上 3–5 cm，於生長至最高分櫱以 200 mM NaCl 鹽水進行鹽分逆境處理。試驗以 RCBD 設計排列，3 重複，每重複 3 株。兩試驗皆於植株間耐鹽性表現出差異時為調查起始日，每日調查至植株死亡為止。

耐鹽品系不同鹽分濃度之調查：利用上述 10 個 BC_2F_3 品系於五葉齡時期進行鹽分逆境處理，並以輪迴親 ‘TK9’ 作為對照品種。使用 37 cm × 27 cm 之 96 孔盤水耕栽培盤進行材料培育，五葉齡階段分別添加 100、150、200、250 及 300 mM 等不同濃度之氯化鈉溶液處理。試驗以 RCBD 設計排列，兩重複，每重複 8 株，於植株表現出差異時開始調查直至植株死亡為止。

分子標幟分析

水稻基因體 DNA 萃取：水稻基因體 DNA 萃取使用 Kazuhiro *et al*. (1998) 發表之 benzyl chloride 法萃取水稻 DNA。分子標幟則利用 Thomson *et al*. (2010) 進行前景選拔所使用的分子標幟及 Hsu (2012) 發表之多型性分子標幟資料，挑選在 ‘Pokkali’ 及 ‘TK9’ 間具有多型性的分子標幟進行分析。

PCR 反應、電泳分析及基因型判讀：PCR 反應流程如下：94℃設定 2 min，循環 1 次；94℃設定 30 s，55℃設定 30 s，72℃設定 30 s，共循環 35 次；72℃設定 2 min，循環 1 次；最後設定在 20℃結束。PCR 增殖產物使用 2.5% SFR 洋菜膠，利用電泳系統在 200 V 下持續 25 min 進行電泳分析。比對親本與子代在 PCR 增殖後的 DNA 片段大小，出現 1 個條帶與 ‘Pokkali’ 品種之條帶相同，則此子代為帶有 ‘Pokkali’ 相同 DNA 之同型結合子，基因型紀錄為 A；出現一個條帶與 ‘TK9’ 之條帶相同，則此子代為帶有 ‘TK9’ 相同 DNA 之同型結合

子，基因型紀錄為 B；若子代同時出現兩個與兩親本相同之 DNA 條帶，則為異型結合子，基因型紀錄為 H。不論在 BC_1F_1 或 BC_2F_1 之回交後代植株，其基因型只會出現同型結合子 B 或異型結合子 H，若出現同型結合子 A 則為自交種子，此株即淘汰。

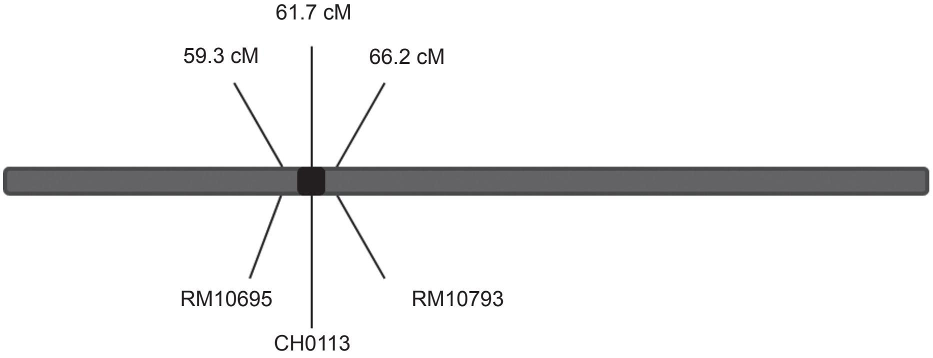
多型性分子標幟篩選：分子標幟的挑選依據 Hsu (2012) 發表之多型性分子標幟資料，挑選輪迴親本 ‘Pokkali’ 與 ‘TK9’ 間具多型性的分子標幟進行分析，並額外自行設計之 SSR 及 Indel 分子標幟填補不足位置。SSR 來源根據水稻分子標幟資料庫網站，Gramene (http://www.gramene.org/markers/microsat/) 已公布的 SSR 分子標幟序列 (Supplementary Table 18, IRGSP, 2005)，Indel 的設計則是根據 Shen *et al*. (2004) 所發表 Indel 存在位置進行相關引設計。至於應用於 *Saltol* QTL 篩選之分子標幟則挑選第一條染色體 59.3–66.2 cM 位置之 3 個分子標幟進行，其中包括與 Saltol 緊密連鎖的 1 個 indel 分子標誌 CH0113 及兩個位於 *Saltol* QTL 兩側之 SSR 分子標幟 RM10793 及 RM10695 進行前景選拔 (圖 3)。

遺傳相似度分析：將基因型判讀結果以 GGT graphical genotype (GGT 2.0)/2007 EDITION 軟體 (http://www.plantbreeding.wur.nl/) 進行統計分析，進一步計算出子代之遺傳背景相似度。圖 4 之連鎖圖譜乃根據上述所篩選出親本間具有多型性的分子標幟在 RAP-DB 網站所公布的 BAC/PAC 位置，換算成 IRGSP (The International Rice Genome Sequencing Project) 網站 Build 5 版公布的 ‘Nipponbare’/‘Kasalath’ 雜交族群的連鎖圖譜位置，並將結果利用 GGT 2.0 繪出之圖譜。

結果

分子標幟輔助水稻耐鹽性選育

分子標幟前景選拔：各世代之秧苗生長至五葉齡時期進行鹽分逆境篩選，存活之植株利用位於第一條染色體 59.3–66.2 cM 與 *Saltol* 緊密連鎖的 1 個 indel 分子標幟 CH0113 及 2 個位於 *Saltol* QTL 兩側之 SSR 分子標幟 RM10793 及 RM10695 (圖 3) 進行前景選拔，經基因型判讀於 BC_1F_1 及 BC_2F_1 世代選取帶有 ‘Pokkali’/‘TK9’ 異型結合子基因型之植株。而 BC_2F_2 則選取帶有 ‘Pokkali’ 同型結合子基因型之植株，選出之植株均進行背景選拔。結果在 BC_1F_1、BC_2F_1 及 BC_2F_2 世代分別共選出 36、54 及 23 株，選出帶有 *Saltol* QTL 區間片段之植株供後續耐鹽特性試驗測試驗利用。



Name	Mb	cM	Notes	Forward primer	Reverse primer
RM10695	10.98	58.1-60.6	flanking marker	ACTCCATGAAACAAACGAAC	TTGAATTCTACTAGTTTGGAAGG
CH0113	11.46	60.9-62.5	foreground *Saltol*	CCCAACGGCACATTAAAATC	GATCCTGAGACCTGACACG
RM10793	12.5	66.2	flanking marker	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC

圖 3. 應用於回交品系之前景選拔所使用之功能性分子標幟及鄰近兩側分子標幟位置與序列。

Fig. 3. The position and sequence of functional markers and flanking markers used in foreground selection of back-cross lines.

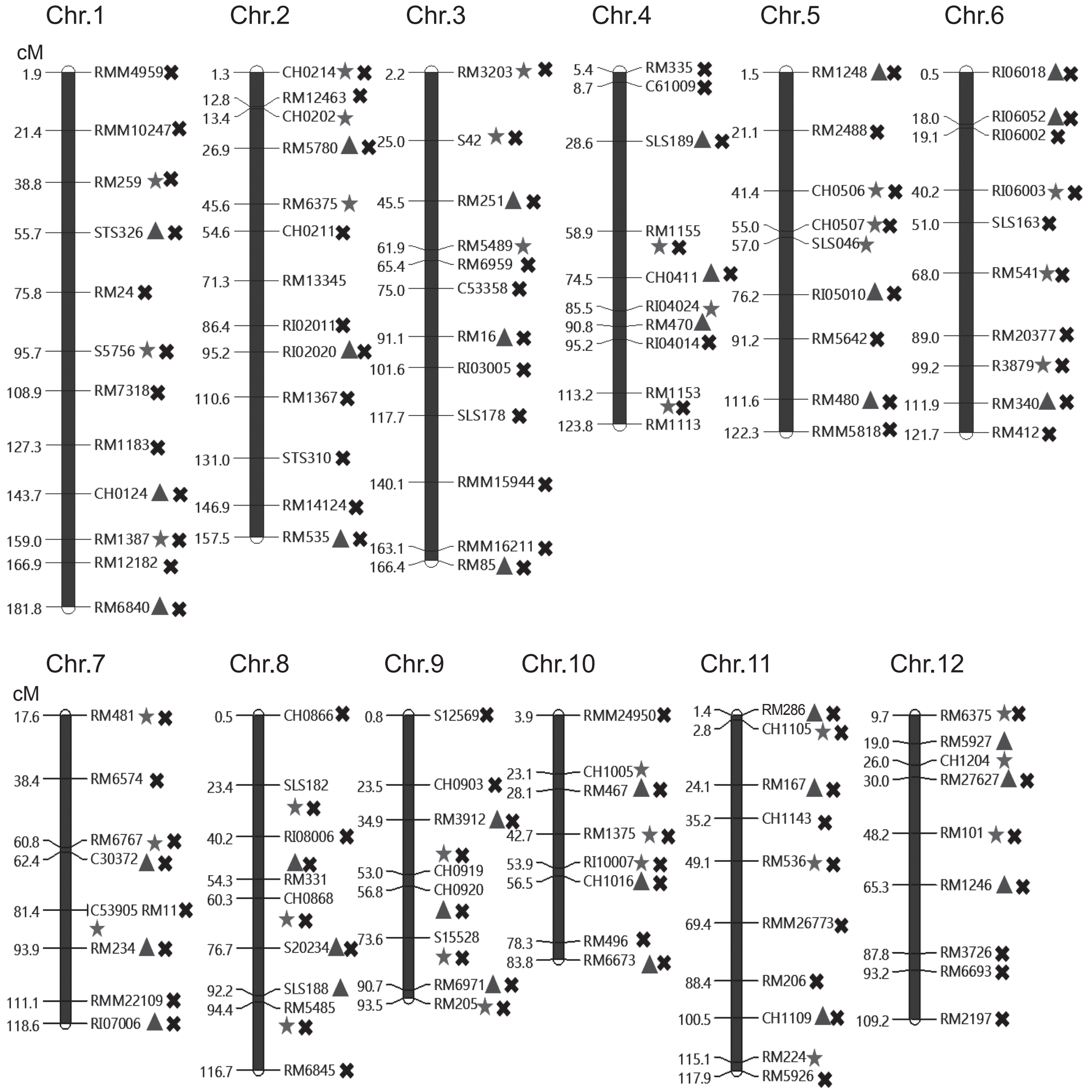


圖 4. 應用於背景選拔之多型性分子標幟。其中 36 個分子標幟於 BC_1F_1 背景選拔 (星形)、36 個分子標幟於 BC_2F_1 背景選拔 (三角形) 及 107 個分子標幟於 BC_2F_2 背景選拔 (X 形)。分子標幟代號 RM 開頭為 SSR 分子標幟，C、R、S 或 E 為 STS 分子標幟，CH、STS、SLS 或 RI 為 indel 分子標幟。

Fig. 4. The polymorphic markers used for background selection. 36 markers indicated by stars at BC_1F_1 generation, 36 markers indicated by triangle at BC_2F_1 generation, and 107 markers indicated by X at BC_2F_2 generation. The prefixes RM indicates SSR markers, C, R, S or E indicates STS markers, and CH, STS, SLS or RI indicates indel markers.

分子標幟背景選拔：利用分布在 12 條水稻染色體上，共 107 個分子標幟進行背景基因型判讀，其中包括 65 個 SSR 分子標幟、9 個 STS 分子標幟及 33 個 indel 分子標幟 (圖 4、表 1)。經耐鹽選拔及分子標幟前景選拔，BC_1F_1、BC_2F_1 及 BC_2F_2 世代分別共選出 36、54 及 23 株。BC_1F_1 植株使用分布於 12 條水稻染色體上之分子標幟 (表 1)，結果顯示 36 株耐鹽 BC1F1 植株與 ‘TK9’ 的遺傳相似度範圍為 56–87%，總體平均值約在 71.5%；選出

表 1. 'Pokkali' 與 'TK9' 間具多型性分子標幟及應用於耐鹽 QTL 導入族群背景選拔之數量。

Table 1. The number of polymorphic markers between 'Pokkali' and 'TK9' used for background selection.

		Genetic marker			
Backcross generation	Chr.	SSR	STS	Indel	Subtotal
BC_1F_1	1	2	1	0	3
	2	1	0	2	3
	3	2	1	0	3
	4	2	0	1	3
	5	0	0	3	3
	6	1	1	1	3
	7	2	1	0	3
	8	1	0	2	3
	9	1	1	1	3
	10	1	0	2	3
	11	2	0	1	3
	12	2	0	1	3
	Total	17	5	14	36
BC_2F_1	1	1	0	2	3
	2	3	0	0	3
	3	3	0	0	3
	4	1	0	2	3
	5	2	0	1	3
	6	1	0	2	3
	7	1	1	1	3
	8	1	1	1	3
	9	2	0	1	3
	10	2	0	1	3
	11	2	0	1	3
	12	3	0	0	3
	Total	22	2	12	36
BC_2F_2	1	9	1	2	12
	2	6	0	5	11
	3	7	2	2	11
	4	4	1	3	8
	5	5	0	3	8
	6	4	1	5	10
	7	6	1	1	8
	8	3	1	4	8
	9	3	2	3	8
	10	5	0	2	7
	11	6	0	3	9
	12	7	0	0	7
	Total	65	9	33	107

遺傳相似度最高前 10 株，與 ‘TK9’ 之遺傳相似度分別為 86.6、85.9、84.4、82.2、81.5、79.3、76.7、76.2、75 及 73.8%，遺傳相似度平均值為 80.1%。獲選之植株均移植至田間，並於回交時作為母本使用，與 ‘TK9’ 回交。BC_2F_1 回交後代經耐鹽且帶有 *Saltol* QTL 篩選中，結果獲得 54 株植株供分子標幟背景選拔，分子標幟選擇與 BC1F1 世代完全不重複的 36 個分子標幟 (圖 4)，以避免相同之基因體片段重複篩選，降低了分子標幟背景選拔效率；背景選拔結果顯示 BC_2F_1 與 ‘TK9’ 間遺傳相似度介於 68–97%，平均值約為 85.4% (圖 5)。選出 10 株與 ‘TK9’ 遺傳相似度較高之植株，其遺傳相似度分別為 96.5、93.5、92.8、92.4、90.3、90.1、89.9、89.6、89.3 及 89%，平均值為 91.3%，此 10 株獲選 BC_2F_1 進行自交。進一步進行 BC_2F_2 後代耐鹽且帶有 Saltol QTL 之前景選拔，結果選出 23 株耐鹽且帶有 *Saltol* QTL 之 BC_2F_2，為達到覆蓋整個水稻基因體的背景選拔效果，因此挑選了 107 個分布於 12 條水稻染色體上的多型性分子標幟 (表 1、圖 4)，以此 107 個分子標幟進行 BC_2F_2 回交後代之基因型判讀。結果顯示此 23 株與 ‘TK9’ 遺傳相似度範圍為 75.65–87.55%，整體平均約為 82.7% (圖 5)，其中遺傳相似度最高者為 87.55% (表 2)。由此結果可知，BC_2F_2 回交後代遺傳背景回復至輪迴親 ‘TK9’ 之比率，相較於 BC_2F_1 有下降的現象。結果顯示，利用分子標幟輔助選拔進行前景選拔，可準確地將目標性狀轉移進入輪迴親。

BC_2F_3 品系不同鹽分濃度下之耐鹽性評估

以獲選之 10 個 BC_2F_3 世代品系於五葉齡期處理 100、150、200、250 及 300 mM 不同濃度之氯化鈉溶液，評估各品系之穩定性，試驗同時增加輪迴親 ‘TK9’ 做為對照比較，結果如表 3 所示。在 100 mM NaCl 溶液處理下，各品系耐鹽等級表現在第 2 日開始出現差異，因此開始調查直至處理後第 22 日 (圖 6)。以 16 株植株表現之耐鹽等級進行平均，結果顯示處理後第 7 日敏感型親本 ‘TK9’ 即表現出耐鹽等級為 5 級，其餘品系表現僅 3–4 級，直至第 9 日所有品系耐鹽等級均達 5 級以上，第 12 日所有品系耐鹽等級則均達 7 級；而變方分析結果顯示在第 4、7、15 及 21 日各品系間之耐鹽等級達顯著差異水準，其中以 STK6、STK7 及 STK11 有較佳耐鹽表現，而 STK1 則是偏向在鹽分逆境前期表現較優良 (表 3)。

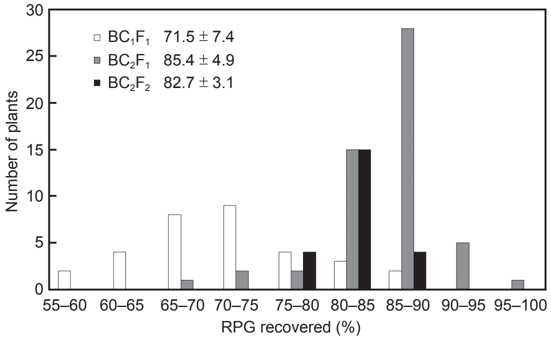


圖 5. ‘Pokkali’/‘TK9’ 分子標幟輔助回交育種後代族群 BC_1F_1、BC_2F_1 及 BC_2F_2 回復輪迴親 ‘TK9’ 基因體比率之頻率分布圖。圖形符號右邊之數值為族群平均輪迴親基因體回復比率。

Fig. 5. The frequency distribution of recurrent parent genome (RPG) recovered rate using marker-assisted backcrossing in BC_1F_1, BC_2F_1, and BC_2F_2 populations derived from the backcross of ‘Pokkali’/‘TK9’. The numbers inside the right side of frame indicate the mean values of RPG recovered.

在 150 mM NaCl 溶液處理下，各品系間耐鹽等級表現在第 2 日開始出現差異，因此開始調查直至處理後第 20 日。結果顯示處理第 8 日後，除 STK1、STK9 及 STK11 外，其餘品系之耐鹽等級皆達 5 級，而敏感型親本 ‘TK9’ 之耐鹽等級則已達到 7 級。至處理第 11 日，除了 STK11 之外，所有品系耐鹽等級均 7 級以上。經變方分析結果顯示，品系間耐鹽等級分別在處理第 6、7、8、9、10、11 及 12 日達到顯著差異水準，其中以 STK7、STK8、STK9 和 STK11 為耐鹽性較佳者，又以 STK11 的耐鹽特性表現最優良 (表 3)。

在 200 mM NaCl 溶液處理下，處理後第 2 日開始調查直至第 16 日。結果顯示處理第 6 日除 STK7 及 STK8 外，其餘品系耐鹽等級均達 5 級，其中耐鹽性表現最佳之品系為 STK7，耐鹽等級平均為 4.8 級，而表現最

表 2. 以 107 個分子標幟應用於 MAS 以分析 'Pokkali' 與 'TK9' 回交後代 BC_2F_2 之基因體組成。

Table 2. The genome composition of BC_2F_2 derived from backcross between 'Pokkali' and 'TK9' by 107 polymorphic markers used for marker-assisted selection.

Individual	N[z] (%)	A[y] (%)	B[x] (%)	H[w] (%)	RPG recovered (%)	Total (cM)	Recombinant[v]	H-segments[u]
TK9	0	0	100	0	100	1,466.5	0	0
3-387	0	8.3	83.4	8.3	87.6	1,466.5	23	6
3-426	0	9.8	84.7	5.5	87.5	1,466.5	21	4
2-171	0	10.5	85.3	4.2	87.4	1,466.5	19	3
3-678	0	12.3	82.5	5.1	85.1	1,466.5	19	2
3-146	0	11.1	80.7	8.2	84.8	1,466.5	20	6
3-178	0	10.9	80.4	8.7	84.8	1,466.5	20	3
210	0	12.4	80.5	7.1	84.1	1,466.5	22	5
4-309	0	9.3	77.3	13.4	84.0	1,466.5	27	8
192	0	13.7	81.2	5.2	83.8	1,466.5	18	3
3-305	0	15.5	81.9	2.6	83.2	1,466.5	19	2
2-124	0	11.2	77.6	11.2	83.2	1,466.5	23	5
268	0	12.5	78.7	8.8	83.1	1,466.5	23	4
126	0	12.4	78.2	9.4	82.9	1,466.5	21	4
2-209	0	8.0	73.1	18.9	82.6	1,466.5	26	8
4-194	0	12.5	77.2	10.3	82.4	1,466.5	23	4
20	0	16.2	80.4	3.4	82.1	1,466.5	21	2
189	0	15.0	78.1	6.9	81.6	1,466.5	21	3
4-271	0	14.2	77.1	8.6	81.4	1,466.5	26	6
219	0	9.2	69.8	21.0	80.3	1,466.5	28	8
151	0	12.6	72.3	15.1	79.9	1,466.5	20	5
4-127	0	17.6	72.9	9.5	77.7	1,466.5	27	6
4-55	0	21.4	74.0	4.6	76.3	1,466.5	24	5
2-37	0	16.7	68.0	15.3	75.7	1,466.5	24	7
Pokkali	0	100	0	0	0	1,466.5	0	0

[z] Variety name (Arrangement by RPG recovered of CNY911303).
[y] The percent of Hang-Yu 15 genome.
[x] The percent of recurrent parent genome.
[w] The percent of heterozygote.
[v] The frequency of cross-over happened in chromosome segment.
[u] Amount of heterozygote in all markers.

差之品系為 STK2，耐鹽等級平均為 6.5，至於敏感型親本 'TK9' 則達 7 級以上。處理第 9 日除 STK4 及 STK8 外，其餘品系耐鹽等級均達 7 級以上，且 'TK9' 之植株均已死亡；經由變方分析顯示處理第 12 日以後各品系間未達顯著性差異水準外，其餘天數皆達顯著水準，其中以 STK8 之耐鹽表現最佳，而 STK4 及 STK7 也有不錯的耐鹽性表現 (表 3)。

在 250 mM NaCl 溶液處理下，各品系間第 2 日葉片即表現出差異，於當日開始進行調查直至第 9 日植株全部死亡為止。試驗結果顯示，鹽分濃度提高到 250 mM NaCl 後，處理第 3 日除 STK7、STK8 和 STK9 外，其餘品系之耐鹽等級表現皆達 5 級，第 5 日所有品系耐鹽等級皆達 7 級，而親本 'TK9' 則大部分植株接近死亡；變方分析結果顯示各品系間在第 2、5、7 及 8 日均達顯著水準，其中發現 STK8 之耐鹽表現最佳，而 STK7 及 STK9 也

表 3. 'TK9' 和 10 個 BC_2F_3 品系於 5 葉齡進行 5 種不同氯化鈉濃度鹽分逆境處理 22 d 之耐鹽等級表現。

Table 3. The scales of salt-tolerance for 'TK9' and 10 BC_2F_3 lines under 5 concentrations of NaCl solution treatments for 22 days at 5-leaf stage.

NaCl conc.	Line or variety	Scale of salt-tolerance[z] Days after treatment 2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	20	21	22
100 mM	STK1	1.0	1.6	2.3 abc	2.6	3.0	3.5 b	4.3	5.75 ab	6.6	7.0	7.8	8.0	8.8 ab	8.6	8.8	9.0	9.0	9.0 a	9.0
	STK2	1.3	2.0	2.6 abc	3.0	3.3	4.5 ab	5.0	6.0 ab	7.0	7.6	8.1	8.5	8.9 ab	8.9	9.0	9.0	9.0	9.0 a	9.0
	STK3	1.1	1.3	1.9 bc	2.8	3.0	4.1 ab	5.3	6.5 ab	6.9	7.4	8.1	8.5	8.8 ab	8.6	8.8	8.8	8.8	9.0 a	9.0
	STK4	1.1	1.3	2.0 bc	2.5	3.3	4.0 ab	4.8	6.0 ab	6.9	7.4	7.8	8.0	8.4 ab	8.5	8.8	8.9	9.0	9.0 a	9.0
	STK6	1.3	1.8	2.9 ab	3.0	3.3	3.4 b	4.6	6.0 ab	6.8	7.8	8.1	8.4	8.9 ab	8.5	8.9	9.0	9.0	9.0 a	9.0
	STK7	1.3	1.8	2.0 bc	2.9	3.0	3.4 b	4.0	5.3 ab	6.4	6.9	7.3	7.9	8.0 b	8.3	8.5	8.5	8.8	8.8	9.0
	STK8	1.0	1.5	2.4 abc	2.8	3.4	3.8 ab	4.5	6.0 ab	6.6	7.0	7.6	8.1	8.5 ab	8.8	8.9	9.0	9.0	9.0 a	9.0
	STK9	1.1	1.4	2.4 abc	3.0	3.1	3.8 ab	4.5	5.0 ab	6.3	6.4	7.5	7.8	8.4 ab	8.6	8.8	8.8	9.0	9.0 a	9.0
	STK10	1.3	1.9	2.6 abc	3.0	3.3	3.6 ab	5.0	6.1 ab	7.3	7.6	8.1	8.4	8.8 ab	8.9	9.0	9.0	9.0	9.0 a	9.0
	STK11	1.1	1.5	1.6 c	2.6	3.3	3.9 ab	4.6	6.1 ab	6.8	7.1	7.6	8.0	8.5 ab	8.6	8.8	8.8	9.0	9.0 a	9.0
	TK9	1.5	1.9	3.1 a	3.1	3.8	5.1 a	5.6	6.9 a	7.8	8.0	8.3	8.4	9.0 a	8.9	8.9	8.9	9.0	9.0 a	9.0
150 mM	STK1	1.1	2.3	2.9	3.0	3.0 c	3.0 bcd	4.8 b	6.0 ab	7.3 ab	7.6 abc	8.1 abc	8.5	8.6	8.9	8.9	8.9	9.0		
	STK2	1.3	2.1	2.9	3.0	3.9 ab	4.8 abc	6.1 ab	6.8 ab	7.6 ab	8.5 a	8.8 ab	8.9	9.0	9.0	9.0	9.0	9.0		
	STK3	1.1	2.5	2.8	3.0	3.6 abc	4.9 bcd	5.9 ab	7.0 ab	7.8 ab	7.9 abc	8.1 abc	8.9	9.0	9.0	9.0	9.0	9.0		
	STK4	1.3	2.1	2.9	3.3	3.5 abc	4.3 bcd	5.4 b	6.6 ab	7.4 ab	7.8 abc	8.1 abc	9.0	9.0	9.0	9.0	9.0	9.0		
	STK6	1.1	2.0	3.0	3.0	3.1 bc	3.6 bcd	5.3 b	6.4 ab	7.3 ab	7.3 abc	7.9 abc	8.6	8.8	9.0	9.0	9.0	9.0		
	STK7	1.3	2.3	2.9	3.0	3.1 bc	3.6 bcd	5.1 b	6.1 ab	6.9 b	7.3 abc	7.6 abc	8.5	8.9	8.9	8.9	8.9	9.0		
	STK8	1.4	2.3	2.8	3.0	3.3 bc	3.5 cd	5.1 b	6.0 ab	6.9 b	7.1 c	8.1 abc	8.9	9.0	9.0	9.0	9.0	9.0		
	STK9	2.0	2.5	2.8	3.0	3.0 c	3.1 d	4.8 b	6.3 ab	6.9 b	7.1 c	7.9 abc	9.0	9.0	9.0	9.0	9.0	9.0		
	STK10	1.5	2.1	3.0	3.0	3.3 bc	4.5 bc	5.5 ab	6.8 ab	7.3 ab	8.0 abc	8.5 ab	8.8	9.0	9.0	9.0	9.0	9.0		
	STK11	1.1	2.1	2.9	3.0	3.0 c	3.1 d	4.8	5.8 b	6.6 b	6.9 c	7.1 c	8.8	8.9	8.9	8.9	9.0	9.0		
	TK9	2.0	2.8	3.0	3.3	4.1 a	5.9 a	7.0 a	7.1 a	8.1 a	8.4 ab	9.0 a	9.0	9.0	9.0	9.0	9.0	9.0		

表 3. ‘TK9’ 和 10 個 BC_2F_3 品系於 5 葉齡進行 5 種不同氯化鈉濃度鹽分逆境處理 22 d 之耐鹽等級表現 (續)。

Table 3. The scales of salt-tolerance for ‘TK9’ and 10 BC_2F_3 lines under 5 concentrations of NaCl solution treatments for 22 days at 5-leaf stage (continued).

NaCl conc.	Line or variety	Scale of salt-tolerance[z]																		
		Days after treatment																		
		2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	20	21	22
200 mM	STK1	2.5 abc	3.0 b	3.6 b	4.3 b	5.8 ab	6.6 abc	6.8 bc	7.3 bc	8.4 ab	8.9 ab	9.0	9.0	9.0	9.0					
	STK2	2.4 abc	3.4 b	3.9 b	4.8 b	6.5 ab	7.1 ab	7.4 ab	8.1 ab	8.6 ab	9.0 a	9.0	9.0	9.0	9.0					
	STK3	2.0 abc	3.3 b	3.9 b	4.9 b	6.1 ab	6.8 abc	7.3 abc	7.9 ab	8.6 ab	8.9 ab	8.9	8.9	9.0	9.0					
	STK4	1.5 c	3.0 b	3.3 b	3.3 b	5.3 b	5.8 bc	6.5 bc	6.5 c	8.3 ab	8.6 ab	8.8	8.9	9.0	9.0					
	STK6	2.6 ab	3.1 b	3.4 b	4.3 b	5.0 b	5.6 bc	7.0 abc	7.8 abc	8.8 ab	8.8 ab	8.9	9.0	9.0	9.0					
	STK7	2.1 abc	3.1 b	3.3 b	3.9 b	4.8 b	5.9 bc	6.4 bc	7.4 bc	8.3 ab	8.4 ab	8.5	9.0	9.0	9.0					
	STK8	2.0 abc	2.8 b	3.5 b	3.9 b	4.9 b	5.5 c	6.1 c	6.5 c	7.9 b	8.1 ab	8.5	8.8	8.9	9.0					
	STK9	1.8 bc	3.4 b	3.8 b	4.5 b	5.5 b	6.1 abc	6.8 bc	7.9 ab	8.3 ab	8.5 ab	9.0	9.0	9.0	9.0					
	STK10	2.4 abc	3.3 b	3.4 b	3.9 b	5.5 b	6.9 abc	7.1 abc	7.6 bc	8.5 ab	8.8 ab	9.0	9.0	9.0	9.0					
	STK11	2.1 abc	3.3 b	3.5 b	4.4 b	5.6 b	6.0 abc	6.8 bc	7.9 ab	8.5 ab	8.9 ab	9.0	9.0	9.0	9.0					
	TK9	2.9 a	4.4 a	6.3 a	7.0 a	7.4 a	7.5 a	8.1 a	9.0 a	9.0 a	9.0 a	9.0	9.0	9.0	9.0					
250 mM	STK1	2.9	5.1 bc	6.9	7.0 b	7.4	7.6 b	8.6 ab	9.0											
	STK2	2.8	5.1 bc	7.0	7.0 b	7.4	8.0 ab	8.6 ab	9.0											
	STK3	3.0	5.8 ab	7.0	7.0 b	7.6	8.1 ab	8.6 ab	9.0											
	STK4	3.0	5.3 bc	6.9	7.0 b	7.3	7.3 b	8.6 ab	9.0											
	STK6	3.0	5.5 ab	7.0	7.0 b	7.4	7.5 b	8.9 a	9.0											
	STK7	2.6	4.8 bc	7.0	7.0 b	7.0	7.4 b	8.5 ab	9.0											
	STK8	2.8	4.4 c	6.6	7.0 b	7.0	7.3 b	8.0 b	9.0											
	STK9	2.8	4.9 bc	6.9	7.0 b	7.6	8.0 ab	9.0 a	9.0											
	STK10	3.0	5.5 ab	7.0	7.0 b	7.9	8.1 ab	9.0 a	9.0											
	STK11	2.9	5.6 ab	7.0	7.0 b	7.8	8.0 ab	9.0 a	9.0											
	TK9	3.0	6.5 a	7.0	7.9 a	8.0	8.8 a	8.9 a	9.0											

表 3. ‘TK9’ 和 10 個 BC_2F_3 品系於 5 葉齡進行 5 種不同氯化鈉濃度鹽分逆境處理 22 d 之耐鹽等級表現 (續)。

Table 3. The scales of salt-tolerance for ‘TK9’ and 10 BC_2F_3 lines under 5 concentrations of NaCl solution treatments for 22 days at 5-leaf stage (continued).

NaCl conc.	Line or variety	Scale of salt-tolerance[z] Days after treatment																		
		2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	20	21	22
300 mM	STK1	3.0	5.5 b	6.8	7.0 b	7.5 ab	8.4 ab	9.0												
	STK2	3.0	5.4 b	7.0	7.0 b	7.3 b	8.8 ab	9.0												
	STK3	3.0	6.1 ab	7.0	7.0 b	8.1 ab	8.8 ab	9.0												
	STK4	3.0	5.6 b	7.0	7.1 b	7.4 ab	8.5 ab	9.0												
	STK6	3.0	5.8 b	6.9	7.0 b	7.8 ab	8.9 a	9.0												
	STK7	3.0	5.3 b	6.9	7.0 b	7.6 ab	8.8 ab	9.0												
	STK8	3.0	5.8 b	6.6	7.0 b	7.3 b	8.0 b	9.0												
	STK9	3.0	5.6 b	7.0	7.0 b	8.0 ab	8.8 ab	9.0												
	STK10	3.0	6.3 ab	7.0	7.0 b	7.9 ab	9.0 a	9.0												
	STK11	3.0	6.3 ab	6.9	7.0 b	7.8 ab	8.9 a	9.0												
	TK9	3.0	7.0 a	7.0	7.9 a	8.4 a	9.0 a	9.0												

[z] Means with the same letter(s) of a row are not significantly different at 5% level by least significant difference (LSD) test.

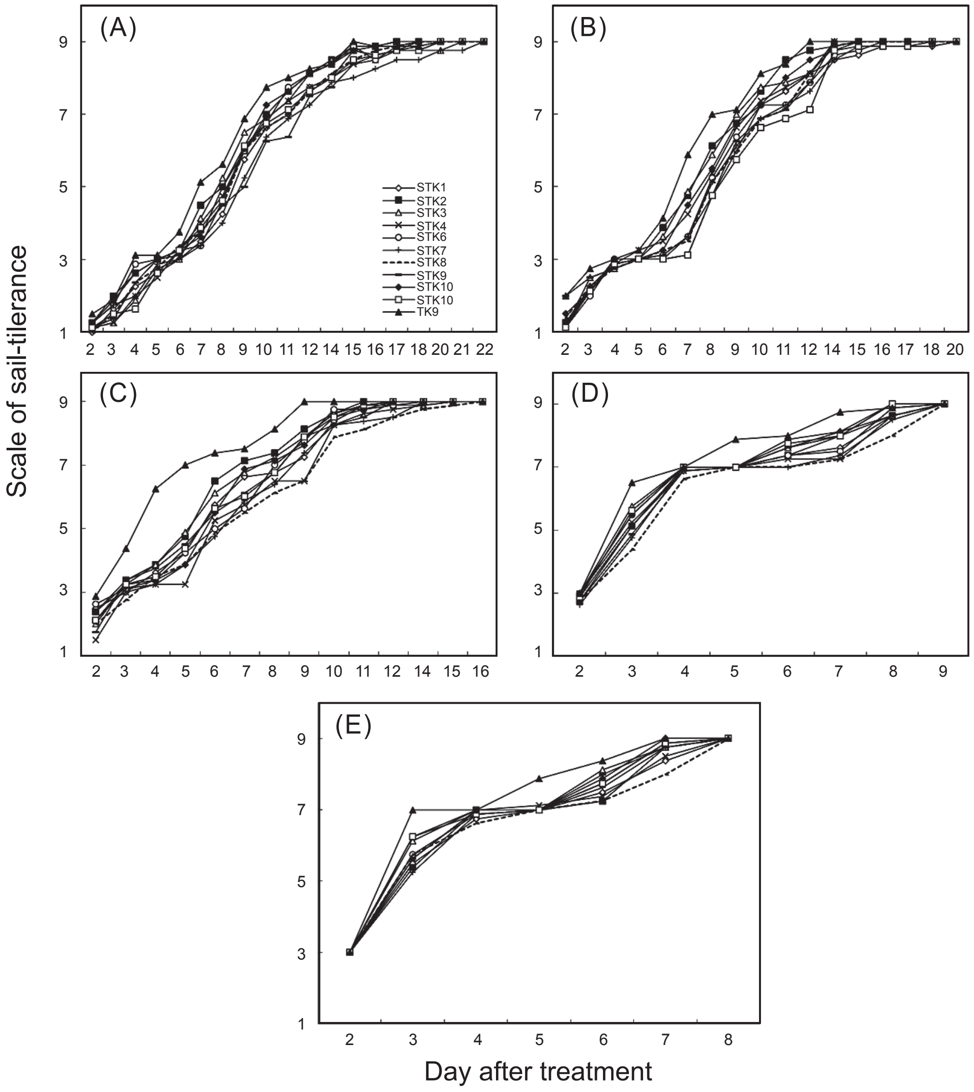


圖 6. 輪迴親 ‘TK9’ 及 10 個 ‘TK9’/‘Pokkali’ 回交後代 BC_2F_3 品系於五葉齡時期進行不同濃度 (A) 100 mM；(B) 150 mM；(C) 200 mM；(D) 250 mM 及 (E) 300 mM 氯化鈉鹽分逆境處理下之耐鹽等級變化。

Fig. 6. The salt-tolerance scales for evaluating recurrent parent ‘TK9’ and 10 BC_2F_3 lines derived from the back-cross of ‘Pokkali’/‘TK9’ at five-leaf seedling stage under five different NaCl concentrations. (A) 100 mM, (B) 150 mM, (C) 200 mM, (D) 250 mM, and (E) 300 mM NaCl.

偏向表現較佳的耐鹽性 (表 3)。

當施以 300 mM NaCl 溶液之鹽分濃度處理，同樣於第 2 日開始進行調查至第 8 日植株全部死亡。結果與 250 mM NaCl 溶液處理下之情況相似，在第 3 日及第 5 日品系之耐鹽等級分別達 5 級及 7 級，而敏感型親本 ‘TK9’ 則是在第 3 日即達耐鹽等級 7 級。經變方分析結果顯示在處理第 3、5 和 6 日品系間耐鹽等級均達顯著水準，結果顯示 STK8 及 STK2 之耐鹽等級表現較佳。綜觀此 11 個耐鹽品系在 5 種不同鹽分濃度下之耐鹽表現，以 STK7 及 STK8 為最優良耐鹽品系，於 4 種鹽分濃度下均表現出較佳的耐鹽性；而 STK2、STK4、STK6、STK9 及 STK11 則為單一濃度下有較佳耐鹽表現者，因此 STK7 及 STK8 為不同鹽分濃度下耐鹽性表現較為穩定的品系 (表 3)。

BC_2F_3 品系不同生長時期下之耐鹽性評估

以 10 個 BC_2F_3 回交世代之品系及輪迴親 ‘TK9’ 於五葉齡及最高分蘗期進行 200 mM NaCl 鹽分逆境處理，藉以了解獲選品系在秧苗期及成株期生長階段鹽分逆境耐受性，結果顯示在五葉齡時期進行 200 mM NaCl 鹽分逆境處理後，其於第 2 日各品種 (系) 間耐鹽等級即出現差異，於此日開始進行調查，直至第 16 日植株全部乾枯死亡時為止，試驗結果如表 4 所示。顯示處理後第 5 日輪迴親 ‘TK9’ 之耐鹽等級即達 7 級，而各品系則於處理第 7 日後，其耐鹽等級方達 5 級以上。至於處理第 9 日除 STK4 及 STK8 外，其餘品種 (系) 耐鹽等級均達 7 級以上，而輪迴親 ‘TK9’ 則全數乾枯死亡。變方分析結果顯示，除處理 12 日以後各品種 (系) 間未達顯著差異水準外，其餘天數皆達顯著差異水準，處理結果顯示品系 STK8 之耐鹽等級表現最佳，而 STK4 及 STK7 之耐鹽性亦有不錯表現 (表 4、圖 7)。

最高分蘗期之各品種 (系) 間於耐鹽處理後第 9 日鹽害情形出現，因此開始進行耐鹽等級調查直至第 30 日，結果如表 4 所示。顯示處理 18 日之前，各品種 (系) 間之耐鹽等級表現皆無顯著差異，處理後第 20 日時，除了 STK7 之外，其餘品種 (系) 耐鹽等級均達 5 級以上。其中輪迴親 ‘TK9’ 之耐鹽等級已達 7 級，與耐鹽表現最優良之品系 STK7 差距為 2.7 級。處理第 28 日後除 STK1 及 STK7 之外，所有品種 (系) 之耐鹽等級均達 7 級以上；變方分析結果顯示品種 (系) 間在處理第 18 日後之耐鹽等級差異始達顯著水準，而多重比較結果顯示品系STK7及STK8之耐鹽表現最佳 (表4、圖7)。

討論

分子標幟輔助水稻耐鹽性之選育

分子標幟輔助回交育種，傳統回交育種在試驗族群規模足夠的狀況下，於 BC_1、BC_2、BC_3 及 BC_4 世代之遺傳背景回復到輪迴親基因型的理論值分別為 75、87.5、93.8 及 96.9%。但在現實育種流程中，大多會因為雜交親本遺傳背景差異大或族群數量不足，使各回交世代遺傳背景回復到輪迴親基因組之比率低於理論值 (Collard *et al*. 2005)。在傳統回交育種操作過程中，一般需回交 6–8 次，回交後代之基因組才能接近於輪迴親基因組，而透過分子標幟輔助回交育種，僅需回交 3–4 次即可達到傳統回交育種的功效。若將分子標幟使用之密度提高到 10 cM 以內，甚至可回交 2 次即可達到目標回復率 (Collard *et al*. 2005; Collard & Mackill 2008; Xu & Crouch 2008)。

本試驗雜交組合在回交 2 次之 BC_2 世代，選出的回交後代遺傳背景與輪迴親基因組的相似度介於 89–96%。然 BC_2F_2 經 107 個分子標幟分析基因組後，顯示遺傳背景相似度僅平均介於 76–88%，遺傳相似度平均值接近 BC_2F_1 的理論值，並較 BC_2F_1 的實際值偏低。其原因可能是因本試驗所選用之雜交親本間的遺傳背景差異較大，或因遺傳拖曳 (genetic drag)、重組、回交族群數量不足等因素，抑或是在 BC_1F_1 及 BC_2F_1 世代選用的分子標幟判讀基因型皆與輪迴親相同。而 BC_2F_2 新加入之分子標幟判讀基因型可能為異型結合或與貢獻親為相同基因型，透過比較 BC_2F_1 及 BC_2F_2 背景選拔之基因型判讀，顯示 BC_2F_2 新增的分子標幟基因型多與貢獻親相同，進而降低回交後代與輪迴親遺傳背景相似度，因此可看出為連鎖拖曳。以上原因，造成 BC_2F_2 遺傳回復率有偏低的現象。由 BC_1F_1 獲選回交後代之基因圖譜 (資料未列) 可發現，這些獲選的回交後代在第 1、3、4、6、11 及 12 條染色體上，其遺傳背景大多未回復到輪迴親之遺傳背景。惟就 BC_1F_1 及 BC_2F_1 遺傳回復率來看，其它水稻耐鹽性方面之研究也有相似的選拔結果。例如 Kuo *et al*. (2013) 利用水稻全基因體上共 100 個分子標幟進行水稻耐鹽性回交育種，於不同回交世代進行遺傳相似度分析，其回復率在 BC_1 回交世代約 75%，BC_2 世代為 89%；Vu *et al*. (2012) 同樣利用分子標幟回交育種法，將 FL478 所帶有的 *Saltol* QTL 堆疊到越南耐鹽品種 BT7，共選用 89 個分子標幟進行背景選拔，於 BC_1F_1 回交世代遺傳相似度範圍為 71–76%，BC_2F_1 回交世代為 88.5–95.5%，

表 4. ‘TK9’ 和 10 個 BC_2F_3 品系於 5 葉齡及最高分蘗期進行 200 mM NaCl 鹽分逆境處理 30 d 之耐鹽等級表現結果。

Table 4. The scale of salt-tolerance for ‘TK9’ and 10 BC_2F_3 lines at 5-leaf stage seedling and maximum tillering stage under the treatment of 200 mM NaCl solution for 30 days.

		Scale of salt-tolerance^z																				
		Days after treatment																				
Stage	Line or variety	2	3	4	5	6	7	8	9	10	11	12	14	15	16	18	20	22	24	26	28	30
Five-leaf stage	STK1	2.5 abc	3.0 b	3.6 b	4.3 b	5.8 ab	6.6 abc	6.8 bc	7.3 bc	8.4 ab	8.9 ab	9.0	9.0	9.0	9.0							
	STK2	2.4 abc	3.4 b	3.9 b	4.8 b	6.5 ab	7.1 ab	7.4 ab	8.1 ab	8.6 ab	9.0 a	9.0	9.0	9.0	9.0							
	STK3	2.0 abc	3.3 b	3.9 b	4.9 b	6.1 ab	6.8 abc	7.3 abc	7.9 ab	8.6 ab	8.9 ab	8.9	8.9	9.0	9.0							
	STK4	1.5 c	3.0 b	3.3 b	3.3 b	5.3 b	5.8 bc	6.5 bc	6.5 c	8.3 ab	8.6 ab	8.8	8.9	9.0	9.0							
	STK6	2.6 ab	3.1 b	3.4 b	4.3 b	5.0 b	5.6 bc	7.0 abc	7.8 abc	8.8 ab	8.8 ab	8.9	9.0	9.0	9.0							
	STK7	2.1 abc	3.1 b	3.3 b	3.9 b	4.8 b	5.9 bc	6.4 bc	7.4 bc	8.3 ab	8.4 ab	8.5	9.0	9.0	9.0							
	STK8	2.0 abc	2.8 b	3.5 b	3.9 b	4.9 b	5.5 c	6.1 c	6.5 c	7.9 b	8.1 ab	8.5	8.8	8.9	9.0							
	STK9	1.8 bc	3.4 b	3.8 b	4.5 b	5.5 b	6.1 abc	6.8 bc	7.9 ab	8.3 ab	8.5 ab	9.0	9.0	9.0	9.0							
	STK10	2.4 abc	3.3 b	3.4 b	3.9 b	5.5 b	6.9 abc	7.1 abc	7.6 bc	8.5 ab	8.8 ab	9.0	9.0	9.0	9.0							
	STK11	2.1 abc	3.3 b	3.5 b	4.4 b	5.6 b	6.0 abc	6.8 bc	7.9 ab	8.5 ab	8.9 ab	9.0	9.0	9.0	9.0							
	TK9	2.9 a	4.4 a	6.3 a	7.0 a	7.4 a	7.5 a	8.1 a	9.0 a	9.0 a	9.0 a	9.0	9.0	9.0	9.0							
Maximum tillering stage	STK1								3.0	3.0		3.2	3.2		3.9	4.8 ab	5.2 ab	5.7 ab	5.9 bc	6.6 ab	6.8	7.4
	STK2								3.0	3.2		3.4	3.4		3.9	5.0 ab	5.9 ab	6.3 ab	6.8 abc	7.4 a	7.9	8.1
	STK3								3.0	3.0		3.4	3.4		4.1	5.7 ab	6.6 a	7.0 a	7.4 ab	7.7 a	8.1	8.1
	STK4								3.2	3.4		3.4	3.7		4.1	4.8 ab	5.9 ab	6.6 a	6.8 abc	7.2 ab	7.4	7.7
	STK6								3.0	3.2		3.2	3.4		3.7	4.8 ab	5.9 ab	6.3 ab	6.8 abc	7.4 a	7.4	7.4
	STK7								3.0	3.0		3.0	3.0		3.2	3.9 b	4.3 b	4.6 b	5.2 c	5.9 b	6.8	7.7
	STK8								3.0	3.0		3.0	3.0		3.9	4.1 b	5.2 ab	5.9 ab	5.9 bc	5.9 b	7.0	8.1
	STK9								3.0	3.0		3.4	3.7		4.3	5.2 ab	6.1 ab	6.6 a	7.0 ab	7.2 ab	7.4	8.1
	STK10								3.2	3.2		3.4	3.7		4.6	5.9 ab	6.8 a	7.0 a	7.2 ab	7.4 a	7.7	7.9
	STK11								3.0	3.0		3.0	3.0		3.4	4.3 ab	5.7 ab	6.1 ab	6.3 abc	6.8 ab	7.0	7.4
	TK9								3.0	3.0		3.7	4.1		4.6	6.3 a	7.0 a	7.4 a	7.7 a	7.7 a	7.9	8.1

^{z} Means with the same letter(s) of a row are not significantly different at 5% level by least significant difference (LSD) test.

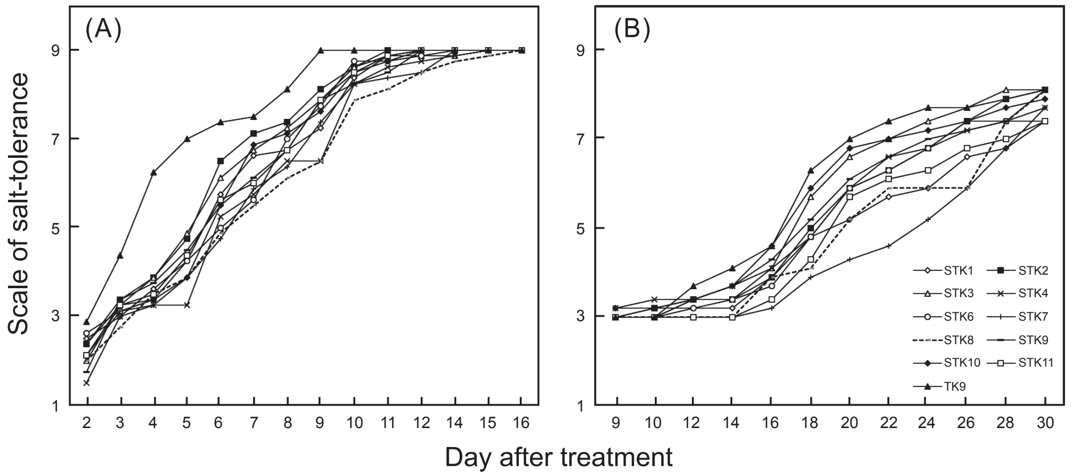


圖 7. 輪迴親 ‘TK9’ 及 10 個 ‘Pokkali’/‘TK9’ 之 BC_2F_3 品系於不同生長時期進行 200 mM 氯化鈉鹽分逆境處理下之耐鹽等級變化。(A) 五葉齡時期；(B) 最高分櫱期。

Fig. 7. The salt-tolerance scales for evaluating recurrent parent ‘TK9’ and 10 BC_2F_3 lines derived from the back-cross of ‘Pokkali’/‘TK9’ at two growth stages under the treatment of 200 mM NaCl solution. (A) 5-leaf seedling stage; (B) Maximum tilling stage.

而 BC_3F_1 回交世代之遺傳相似度最高可達 100%。這些研究結果與本試驗相近，然本試驗回交後代經自交繁殖，結果發現 BC_2F_2 與輪迴親之遺傳背景相似度有下降的現象，未來應直接評估獲選優良耐鹽品系在農藝特性和品質特性之表現，若已符合實際栽培需求，獲選品系將可進一步供鹽分地實際栽培利用測試後，並於命名為新品種後提供台灣水稻鹽化土地栽培利用；若獲選品系在實際栽培表現上未達理想，則仍需再進一步行回交藉以提高遺傳相似度。

BC_2F_3 品系於不同鹽分濃度及不同生長期之耐鹽性評估

不同鹽分濃度對水稻會有不同的傷害，而不同生長時期對鹽分逆境會有不同的反應，許多研究也針對與鹽分逆境相關的各種性狀及不同生長期進行檢測。如 Wang *et al*. (2012) 探討在不同氯化鈉濃度下，水稻地下部及地上部的 Na^+ 與 K^+ 含量以及耐鹽比率；Majeed *et al*. (2013) 探討不同鹽分濃度對發芽率、發芽時間、地上部及地下部乾重和耐鹽指數。這些結果顯示隨著鹽分濃度提高，逆境強度也會增強，而種子發芽率、發芽速度、地上部根長、乾重、地下部根長及乾重等性狀都會隨著鹽分濃度提升而下降，而植株內部 Na^+ 含量更因溶液中 Na^+ 濃度的提升，致使植株吸收更多 Na^+ 離子，進而造成離子毒害。Thomson *et al*. (2010) 針對水稻秧苗期的株高、地上部 Na^+、K^+ 含量、Na^+/K^+ 比率、地下部 Na^+、K^+ 及 Na^+/K^+ 比率等性狀進行 QTL 檢測，顯示第 1 條染色體上 *Saltol* QTL 區間對控制 Na^+/K^+ 比率有所貢獻，而 *Saltol* QTL 證實於水稻秧苗期之耐鹽性有增進的效果。Kumar *et al*. (2015) 利用 GWAS 檢測與成株期相關的 QTL，結果發現第 1 條染色體 *Saltol* QTL 區間的位置上有高度顯著的貢獻，亦即證實 *Saltol* QTL 不僅僅在水稻秧苗期的耐鹽性上有所貢獻，也在水稻成株期的耐鹽性有貢獻。

本試驗雖僅針對耐鹽評估等級進行篩選，但仍獲得相似實驗結果，隨著 NaCl 濃度的提高，其耐鹽等級的表現值上升幅度亦相當劇烈，植株存活天數亦隨著濃度提高而大幅遞減；在不同 NaCl 濃度下品系平均耐鹽等級達 5 級及 7 級的日數亦隨著濃度的上升而減少。究其原因，本次試驗利用不同鹽分濃度對鹽分逆境進行模擬，由於不同鹽分濃度對植株造成的傷害有所差異，在低濃度下鹽害對植株造成

的損傷較為輕微，植株在逆境下尚可維持部分生長發育功能或是修復損傷。而在高鹽分濃度下，參試材料之生長及發育嚴重受阻，亦無法進行損傷修復，因此在處理第 7 至 8 日後所有品系皆死亡。秧苗期及成株期之耐鹽性評估，結果顯示耐鹽等級在 5 葉齡時期時並無劇烈上升的現象，而在成株期，由於植株已生長至成熟株，在鹽分逆境處理前期並無明顯鹽害，品系間也無顯著差異。直至處理第 18 日品系間開始出現顯著差異，且處理達 28 日時大部分品系皆已死亡或 7 級趨近於死亡。

依據 11 個品種 (系) 的耐鹽表現來看，不同鹽分濃度處理下，結果顯示 STK7 及 STK8 兩個品系在耐鹽性上有較為優越的表現，較之對照品種 ‘TK9’ 表現均有顯著性差異存在。在 5 葉齡及最高分櫱期後的鹽分逆境處理結果，在 5 葉齡時期 STK5、STK7 及 STK8 等 3 個品系在耐鹽性上的表現優於輪迴親 ‘TK9’。顯示導入 *Saltol* QTL 對回交後代的耐鹽性有提高的現象，而在最高分櫱期後的鹽分逆境處理，於處理前期品系間皆無明顯差異，然處理第 18 天後各品系間差異即達顯著差異，其中 STK1、STK7 及 STK8 等 3 個獲選耐鹽品系有較佳的耐鹽表現，並較其他品系及輪迴親 ‘TK9’ 間之耐鹽性有顯著差異表現。其中 STK7 及 STK8 回交後代，分別在成株及秧苗期表現良好的耐鹽性。顯示回交品系導入貢獻親 ‘Pokkali’ 所帶有的 *Saltol* QTL 後，由於回交品系帶有維持 Na^+/K^+ 比率之 QTL，使回交品系在鹽分逆境下較不會受鹽分逆境的影響，並提升了特定生育期之耐鹽性，因此使回交品系之耐鹽性有所提升。由於結果顯示導入 *Saltol* QTL 造成品系間在秧苗期及成株期之耐鹽性表現不同，推測雖然 *Saltol* QTL 可同時提升秧苗期及成株期之耐鹽性外，可能尚有其它與耐鹽性相關 QTL 的影響，其隨著回交操作而導入回交後代中 (Thomson *et al*. 2010; Sun *et al*. 2014)，從而某些只在特定生育期之耐鹽性表現佳。如 STK4 僅在秧苗期表現出耐鹽性，而部份品系則全生育期均有良好之耐鹽性 (Ghomi *et al*. 2013; Hossain *et al*. 2015)，如 STK8 及 STK7 在秧苗期及成株期均表現出耐鹽性，使得帶有 Saltol QTL 回交品系在秧苗期及成株之耐鹽性出現了不同程度之差異。

本試驗主要針對水稻於不同鹽分濃度及不同生長期的耐鹽性進行評估，惟 *Saltol* QTL 對耐鹽性的提升有限，後續可將獲選優良回交耐鹽品系繼續純化或導入更多耐鹽相關基因於同一品種。而全生育期均表現耐鹽性之品系由於秧苗期及成株期均可忍受鹽化土壤的逆境，未來於鹽化土壤的利用上極具潛力，應可進一步開發利用以擴大水稻生產面積，藉此培育出耐鹽且同時具有穩產特性之水稻新品種以增進糧食供應及確保糧食安全生產。

引用文獻

Cheng, L., Y. Wang, L. Meng, X. Hu, Y. Cui, Y. Sun, L. Zhu, J. Ali, J. Xu, and Z. Li. 2012. Identification of salt-tolerant QTLs with strong genetic background effect using two sets of reciprocal introgression lines in rice. Genome 55:45–55.

Collard, B. C. and D. J. Mackill. 2008. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Philos. Trans. R. Soc. B Biol. Sci. 363:557–572.

Collard, B. C. Y., M. Z. Z. Jahufer, J. B. Brouwer, and E. C. K. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker assisted selection for crop improvement: The basic concepts. Euphytica 142:169–196.

Darwish, E., C. Testerink, M. Khalil, O. El-Shihy, and T. Munnik. 2009. Phospholipid signaling responses in salt-stressed rice leaves. Plant Cell Physiol. 50:986–997.

Ghomi, K., B. Rabiei, H. Sabouri, and A. Sabouri. 2013. Mapping QTLs for traits related to salinity tolerance at seedling stage of rice (*Oryza sativa* L.): An agrigenomics study of an Iranian rice population. OMICS. 17:242–251.

Gregorio, G. B., D. Senadhira, and R. D. Mendoza. 1997. Screening Rice for Salinity Tolerance. International Rice Research Institute. Manila, Philippines. 30 pp.

Hossain, H., M. A. Rahman, M. S. Alam, and R. K. Singh. 2015. Mapping of quantitative trait loci associated with reproductive-stage salt tolerance in rice. J Agron. Crop Sci. 201:17–31.

Hsu, J. H. 2012. The Platform of Polymorphic Markers and Genetic Mapping of a Drought Tolerance Gene at Seedling Stage in Rice. Master Thesis, Graduate Institute of Agronomy, National Chiayi University.

Chiayi, Taiwan. 98 pp. (in Chinese with English abstract)

Hu, J., M. Cheng, G. Gao, Q. Zhang, J. Xiao, and Y. He. 2013. Pyramiding and evaluation of three dominant brown planthopper resistance genes in the elite indica rice 9311 and its hybrids. Pest Manage. Sci. 69:802–808.

International Rice Genome Sequencing Project. 2005. The map-based sequence of the rice genome. Nature 436:793–800.

Islam, M. R. and G. B. Gregorio. 2013. Progress of salinity tolerant rice variety development in Bangladesh. SABRAO J. Breed. Genet. 45:21–30.

Jiang, H., Y. Feng, L. Bao, X. Li, G. Gao, Q. Zhang, J. Xiao, C. Xu, and Y. He. 2012. Improving blast resistance of Jin 23B and its hybrid rice by marker-assisted gene pyramiding. Mol. Breed. 30:1679–1688.

Kazuhiro, K., Y. Niwa, T. Yamaguchi, H. Sunohara, H. Hirano, and M. Umeda. 1998. A rapid and easy-handling procedure for isolation of DNA from rice, *Arabidopsis* and tobacco. Plant Biotechnol. 15:45–48.

Kumar, V., A. Singh, S. V. A. Mithra1, S. L. Krishnamurthy, S. K. Parida, S. Jain, K. K. Tiwari, P. Kumar, A. R. Rao, S. K. Sharma, J. P. Khurana, N. K. Singh, and T. Mohapatra. 2015. Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). DNA Res. 22:133–145.

Kuo, S. C., C. W. Kuo, Y. R. Lin, and Y. P. Wu. 2013. Marker-assisted selection of salt tolerance in rice (*Oryza sativa* L.). J. Taiwan Agric. Res. 62:137–156. (in Chinese with English abstract)

Majeed, K. A., A. S. Ali, H. H. Hasan, and R. H. Ghal. 2013. Salt tolerance study of six cultivars of rice (*Oryza sativa* L.) during germination and early seedling growth. J. Agric. Sci. 5:1916–9752.

Munns, R. 2005. Genes and salt tolerance: Bringing them together. New Phytol. 167:645–663.

Munns, R. and R. A. James. 2003. Screening methods for salinity tolerance: A case study with tetraploid wheat. Plant Soil 253:201–218.

Neeraja, C. N., R. Maghirang-Rodriguez, A. Pamplona, S. Heuer, B. C. Collard, E. M. Septiningsih, G. Vergara, D. Sanchez, K. Xu, A. M. Ismail, and D. J. Mackill. 2007. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. Theor. Appl. Genet. 115:767–776.

Negrão, S., B. Courtois, N. Ahmadi, I. Abreu, N. Saibo, and M. M. Oliveira. 2011. Recent updates on salinity stress in rice: From physiological to molecular responses. Crit. Rev. Plant Sci. 30:329–377.

Pradhan, S. K., D. K. Nayak, S. Mohanty, L. Behera, S. R. Barik, E. Pandit, S. Lenka, and A. Anandan. 2015. Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. Rice 8:19.

Ren, Z. H., J. P. Gao, L. G. Li, X. L. Cai, W. Huang, D. Y. Chao, M. Z. Zhu, Z. Y. Wang, S. Luan, and H. X. Lin. 2005. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat. Genet. 37:1141–1146.

Ruengphayak, S., E. Chaichumpoo, S. Phromphan, W. Kamolsukyunyong, W. Sukhaket, E. Phuvanartnarubal, S. Korinsak, S. Korinsak, and A. Vanavichit. 2015. Pseudo-backcrossing design for rapidly pyramiding multiple traits into a preferential rice variety. Rice 8:7.

Salgotra, R. K., B. B. Gupta, R. Millwood, M. Balasubramaniam, and C. N. Stewart, Jr. 2012. Introgression of bacterial leaf blight resistance and aroma genes using functional marker-assisted selection in rice (*Oryza sativa* L.). Euphytica 187:313–323.

Shen, Y. J., H. Jiang, J. P. Jin, Z. B. Zhang, B. Xi, Y. Y. He, G. Wang, C. Wang, L. Qian, X. Li, Q. B. Yu, H. J. Liu, D. H. Chen, J. H. Gao, H. Huang, T. L. Shi, and Q. B. Yu. 2004. Development of genome-wide DNA polymorphism database for map-based cloning of rice genes. Plant Physiol. 135:1198–1205.

Singh, R. K. and B. Mishra. 2004. Role of central soil salinity research institute in genetic improvement of rice varieties in India. p.189–242. *in*: Genetic Improvement of Rice Varieties in India. (Sharma, S. D. and U. P. Rao, eds.) Today & Tomorrows Printers and Publishers. New Delhi, India. 1251 pp.

Sun, J., D. T. Zou, F. S. Luan, H. W. Zhao, J. G. Wang, H. L. Liu, D. W. Xie, D. Q. Su, J. Ma, and Z. L. Liu. 2014. Dynamic QTL analysis of the Na^+ content, K^+ content, and Na^+/K^+ ratio in rice roots during the field growth under salt stress. Biol. Plant. 58:689–696.

Swamy, B. P. M. and A. Kumar. 2013. Genomics-based precision breeding approaches to improve drought tolerance in rice. Biotechnol. Adv. 31:1308–1318.

Tavakkoli, E., P. Rengasamy, and G. K. Mcdonald. 2010. High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. J. Exp. Bot. 61:4449–4459.

Thomson, M. J., M. de Ocampo, J. Egdane, M. A. Rahman, A. G. Sajise, D. L. Adorada, E. Tumimbang-Raiz, E. Blumwald, Z. I. Seraj, R. K. Singh, G. B. Gregorio, and A. M. Ismail. 2010. Characterizing the *saltol* quantitative trait locus for salinity tolerance in rice. Rice 3:148–160.

Vu, H. T. T., D. D. Le, A. M. Ismail, and H. H. Le. 2012. Marker-assisted backcrossing (MABC) for improved

salinity tolerance in rice (*Oryza sativa* L.) to cope with climate change in Vietnam. Aust. J. Crop Sci. 6:1649–1654.

Wang, W., B. Vinocur, and A. Altman. 2003. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. Planta 218:1–14.

Wang, Y., H. Guo, H. Li, H. Zhang, and X. Miao. 2012. Identification of transcription factors potential related to brown planthopper resistance in rice via microarray expression profiling. BMC Genom. 13:687.

Xu, Y. and J. H. Crouch. 2008. Marker-assisted selection in plant breeding: From publications to practice. Crop Sci. 48:391–407.

Yamaguchi, T. and E. Blumwald. 2005. Developing salt-tolerant crop plants: Challenges and opportunities. Trends Plant Sci. 10:615–620.

Yoshida, S., D. A. Forno, J. H. Cook, and K. A. Gomez. 1976. Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute. Philippines. 83 pp.

The Introgression of *Saltol* QTL into 'TK9' to Increase Salinity Tolerance by Using Marker-Assisted Selection

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Abstract

Wen, N. H., C. W. Kuo, S. C. Kuo, Y. R. Lin, and Y. P. Wu. 2016. The introgression of *Saltol* QTL into 'TK9' to increase salinity tolerance by using marker-assisted selection. J. Taiwan Agric. Res. 65(3):328–347.

In this study, *japonica* cv. 'TK9', a rice variety with high quality in Taiwan, was used as the recurrent parent and *indica* cv. 'Pokklali', a salt-tolerant variety with *Saltol*, was used as the donor parent. Marker-assisted backcross was applied to introgress *Saltol* QTL from 'Pokkali' to 'TK9'. For each backcross generation, the foreground selection was conducted first, and the selected progenies were subsequently subjected to background selection by marker-assisted selection. Among the backcross progeny carrying *Saltol* QTL, the average recovered rates of genetic background of the recurrent parent in BC_1F_1, BC_2F_1, and BC_2F_2 generations were 71.5, 85.4, and 82.7%, respectively. In comparison with the theoretical value of traditional backcross breeding of BC2F1, 87.5%, the recurrent parent genome recovered rate of BC_2F_2 has decreased, indicating the genetic background between parents were quite different or the condition of genetic dragging occurred. In addition, 'Pokkali', CWY981126, 'IR64', and ten lines of BC_2F_3 were treated with five concentrations of saline soution, 100, 150, 200, 250, and 300 mM of NaCl, at the fifth-leaf seedling stage. The results showed that 'Pokkali' expressed high salt-tolerance under 100, 150, and 200 mM NaCl. Moreover, STK7 and STK8, the backcross progenies of BC2F3, performed better salt-tolerance. Under the condition of 200 mM NaCl, STK8 had the best salt tolerance, followed by STK4 and STK7, at the fifth-leaf stage. STK7 and STK8 had best salt tolerance at active tillering stage. The *Saltol* QTL was successfully introgressed into 'TK9' by the evidence of salinity tolerance in the selected backcross progenies. The backcross lines of BC2F3 with good salinity tolerance have great potential to be developed into cultivars for growing in salt affected areas in Taiwan in the future.

Key words: Marker-Assisted Selection, Salt tolerance, Rice.

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